1982

cancer treatment reports

March 1982
Volume 66
Number 3
Pages 417-599

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service National Institutes of Health

cancer treatment reports

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The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through January 31, 1983.

Contents

Cancer Treatment Reports
Volume 66, Number 3
March 1982

Page

ARTICLES

Current Controversies in Cancer Management

Deferral of Initial Therapy for Advanced Indolent Lymphomas 417
Carol S. Portlock
Yale University, New Haven, Connecticut

Role of Combined-Modality Therapy in the Curative Management of Advanced-Stage "Favorable" Non-Hodgkin's Lymphomas 421
Timothy J. Kinsella
National Cancer Institute, Bethesda, Maryland

Special Features

Streptozocin: A Review of Its Pharmacology, Efficacy, and Toxicity 427
Raymond B. Weiss
National Cancer Institute, Bethesda, Maryland

Rationale for the Use of Alternating Non-Cross-Resistant Chemotherapy 439
J. H. Goldie, A. J. Coldman, and G. A. Gudauskas
Cancer Control Agency of British Columbia, Vancouver, Canada

Clinical Studies

Multiple Myeloma Resistant to Melphalan: Treatment With Doxorubicin, Cyclophosphamide, Carmustine (BCNU), and Prednisone 451
Robert A. Kyle, Thomas F. Pajak, Edward S. Henderson, Ismat U. Nawabi, Kurt Brunner, Patrick H. Henry, O. Ross McIntyre, and James F. Holland
Cancer and Leukemia Group B

Hemolytic-Uremic Syndrome Associated With Mitomycin Therapy 457
Michael D. Pavy, Elizabeth L. Wiley, and Martin D. Abeloff
The Johns Hopkins Oncology Center, Baltimore, Maryland

Tumor Size, Response Rate, and Prognosis in Small Cell Carcinoma of the Bronchus Treated by Combination Chemotherapy 463
P. G. Harper, R. L. Souhami, S. G. Spiro, D. M. Geddes, M. Guimaraes, F. Fearon, and J. F. Smyth
University College Hospital, London, England

Page

Clinical Trial of Cisplatin and 5-FU Infusion as Initial Treatment for Advanced Squamous Cell Carcinoma of the Head and Neck 471
Julie Kish, Anibal Drelichman, John Jacobs, Judith Hoschner, Jeannie Kinzie, John Loh, Arthur Weaver, and Muhyi Al-Sarraf
Wayne State University, Detroit, Michigan

Influence of Renal Failure on Myelosuppressive Effects of Melphalan: Cancer and Leukemia Group B Experience 475
G. G. Cornwell, III, T. F. Pajak, O. R. McIntyre, S. Kochwa, and H. Dosik
Cancer and Leukemia Group B

Cardiac Abnormalities After AMSA Administration 483
Laurel J. Steinherz, Peter G. Steinherz, Deborah Mangiacasale, Charlotte Tan, and Denis R. Miller
Memorial Sloan-Kettering Cancer Center, New York, New York

Combined Radical Radiation Therapy and Chemotherapy for Primary Squamous Cell Carcinoma of the Anal Canal 489
B. J. Cummings, W. D. Rider, A. R. Harwood, T. J. Keane, G. M. Thomas, C. Erlichman, and S. Fine
The Princess Margaret Hospital, Toronto, Ontario, Canada

High-Dose Doxorubicin: An Exploration of the Dose-Response Curve in Human Neoplasia 493
Richard H. Wheeler, William D. Ensminger, James H. Thrall, and Jeffery L. Anderson
University of Michigan, Ann Arbor

Clinical and Preclinical Pharmacology

Comparative Effects of Methotrexate, Two Nonclassic Folic Acid Antagonists, and Cytarabine on Hematopoietic Precursor Cells 499
John C. Marsh
Yale University, New Haven, Connecticut

Effect of Vinblastine, Vincristine, and Vindesine on Neutrophil Phagocytosis and Iodination 505
Veronica Chohan and Raymond M. Lowenthal
University of Tasmania, Hobart, Australia

Studies on the Pharmacokinetics and Metabolism of *cis*-Dichloro-*trans*-dihydroxy-bis-isopropylamine platinum(IV) in the Dog 509
Lakshmi Pendyala, J. Wayne Cowens, and Patrick J. Creaven
Roswell Park Memorial Institute, Buffalo, New York

Enhanced Lymph Node Uptake of Melphalan Following Liposomal Entrapment and Effects on Lymph Node Metastasis in Rats 517
Juneji Khato, Elaine R. Priester, and Susan M. Sieber
National Cancer Institute, Bethesda, Maryland

Page

Serial Analysis of Melanoma Growth Kinetics in a Patient Receiving Bleomycin and Procarbazine Therapy: Comparison of Tumor Volume, Flow Cytometry, and Thymidine Labeling Techniques 529
James J. Stragand, Benjamin Drewinko, Barthel Barlogie, Nicholas Papadopoulous, and Robert A. White
The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

Effect of Cytarabine on Cytotoxic T-Cell Development 535
Vincent J. Merluzzi, Brian M. Susskind, and Ronald B. Faanes
Sloan-Kettering Institute for Cancer Research, Rye, New York

BRIEF REPORTS

Randomized Phase II Comparison of Methotrexate With or Without L-Asparaginase in Non-Small Cell Cancer of the Lung 541
Seth A. Rudnick, Eric Neijstrom, Robert L. Capizzi, David Delaney, and Mark Kirsch
University of North Carolina, Chapel Hill

Response of Hypereosinophilic Syndrome to 6-Thioguanine and Cytarabine 545
Daryl L. Eakin, David P. Gill, and Gary B. Weiss
University of Texas Medical Branch, Galveston

Radiation Therapy Combined With Intra-Arterial 5-FU Chemotherapy for Treatment of Localized Adenocarcinoma of the Pancreas: A Southwest Oncology Group Study 549
Joseph D. McCracken, Marvin Olson, A. B. Cruz, Jr, Lawrence Leichman, and Noboru Oishi
Southwest Oncology Group

Extensive Adenocarcinoma and Large Cell Undifferentiated Carcinoma of the Lung Treated With 5-FU, Vindesine, and Mitomycin (FEMi): A Southwest Oncology Group Study 553
Thomas P. Miller, James K. Weick, Petre N. Grozea, and David A. Carlin
Southwest Oncology Group

Phase II Trial of Razoxane (ICRF-159) in Patients With Squamous Cell Carcinoma of the Head and Neck Previously Exposed to Systemic Chemotherapy 557
M. K. Shah, P. F. Engstrom, R. B. Catalano, A. R. Paul, R. E. Bellet, and R. H. Creech
Fox Chase Cancer Center, Philadelphia, Pennsylvania

Third-Line Chemotherapy With Mitomycin, Vinblastine, and Carmustine (BCNU) in Refractory Breast Carcinoma: A Pilot Study 559
Andrea Veronesi, Umberto Tirelli, Enzo Galligioni, Mauro G. Trovò, Diana Crivellari, M. Donatella Magri, Mario Roncadin, Salvatore Tumolo, and Eligio Grigoletto
Ospedale Civile, Pordenone, Italy

Page

5-Azacitidine and Zorubicin for Patients With Previously Treated Acute Nonlymphocytic Leukemia: A Cancer and Leukemia Group B Pilot Study 563
Bruce A. Peterson, Clara D. Bloomfield, Arlan J. Gottlieb, Morton Coleman, and Mortimer S. Greenberg
Cancer and Leukemia Group B

Phase II Study of the Combination of Vinblastine, Bleomycin, and Cisplatin in Advanced Malignant Melanoma 567
Edward T. Creagan, David L. Ahmann, Allan J. Schutt, and Stephanie J. Green
Mayo Clinic, Rochester, Minnesota

Cerebrospinal Fluid Versus Serum Concentrations of 5-FU, Allopurinol, and Oxypurinol During Treatment of Metastatic Brain Cancer With 5-FU Infusion, Allopurinol, and Radiation 571
C. L. Hornbeck, R. A. Floyd, J. E. Byfield, J. C. Griffiths, S. Frankel, and T. R. Sharp
Veterans Administration Medical Center, San Diego, and University of Southern California School of Pharmacy, Los Angeles

Combination Chemotherapy for the 9L Rat Brain Tumor Model System 575
Tribhawan S. Vats, Bruce F. Kimler, Sheri D. Henderson, and Robert A. Morantz
University of Kansas Medical Center, Kansas City

CLINICAL TRIALS SUMMARIES

A Pilot Study of Estracyt in Advanced Breast Cancer 581
P. J. D. K. Dawes
Newcastle General Hospital, Newcastle Upon Tyne, England

Phase II Evaluation of Vindesine for Patients With Advanced Colorectal Carcinoma 583
J. H. Saiers, M. Slavik, and D. R. McKinney
Veterans Administration Medical Center, Albuquerque, New Mexico

Phase II Trial of Thioproline in Advanced Epidermoid Head and Neck Tumors 585
Francesco Boccardo, Angelo Barbieri, Luciano Canobbio, Domenico Guarneri, Marco Merlano, and Riccardo Rosso
Istituto Scientifico per lo Studio e la Cura dei Tumori, Genoa, Italy

LETTERS

Vindesine-Associated Angina and ECG Changes 587
Richard S. Yancey and Moshe Talpaz
The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston

Page

Contamination of Marihuana Cigarettes With Pathogenic Bacteria—Possible Source of Infection in Cancer Patients 589
J. Thomas Ungerleider, Therese Andrysiak, Donald P. Tashkin, and Robert Peter Gale
University of California, Los Angeles

Cytarabine in Chronic T-Cell Neoplasms 591
George A. Omura
University of Alabama, Birmingham

Enhanced Bleomycin Toxicity During Acute Renal Failure 592
David J. Perry, Raymond B. Weiss, and H. Grant Taylor
Walter Reed Army Medical Center, Washington, DC

AMSA Therapy for Children With Lymphoblastic Malignancy 593
John J. Hutter, Jr, and Frank L. Meyskens
University of Arizona Health Sciences Center, Tucson

Allergic Reaction Following Administration of AMSA 594
Fred P. Rosenfelt, Barry E. Rosenbloom, and Irwin M. Weinstein
Cedars-Sinai Medical Center, Los Angeles, California

ANNOUNCEMENTS 597

Eighth Annual Symposium on Diagnosis and Treatment of Neoplastic Disorders—Medical, Surgical, and Radiotherapeutic Aspects
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Deferral of Initial Therapy for Advanced Indolent Lymphomas [1]

Carol S. Portlock [2,*]

Why is the management of advanced indolent lymphomas[3] controversial? Surely it is because these diseases are not yet known to be curable, while paradoxically they remain highly treatable with long survival. Like patients with their aggressive counterpart, diffuse histiocytic lymphoma, most patients with advanced indolent lymphomas achieve pathologically documented complete remissions with systemic therapy. However, unlike patients with diffuse histiocytic lymphoma, pathologically documented complete responders with indolent lymphomas do not remain in complete remission. Their relapse-free survival curve shows a pattern of continuous late relapse, with no suggestion of plateau (or "cure"). Even the most optimistic report in the literature regarding combination chemotherapy (the use of C-MOPP in NML) has recently been shadowed by relapses as late as 6 years following therapy (1), and a randomized trial comparing C-MOPP to other drug combinations has shown a pattern of continuous late relapse in all three treatment arms (2).

If there is no known curative regimen for patients with stage III and IV indolent lymphomas, is it necessary to initiate treatment at all? In spite of pathologically documented advanced disease at presentation, these patients are often symptomatically well with no disease sites that are threatening. Furthermore, it is well-known that patients with indolent lymphomas, unlike patients with aggressive lymphomas, may have disease which is only slowly progressive or even waxes and wanes without therapy. The potential benefits of withholding initial therapy are severalfold: (*a*) the patient may experience a prolonged treatment-free interval; (*b*) during this interval, the lymphoma will not be exposed to agents which might select for drug resistance; (*c*) the patient may experience spontaneous regression of disease; (*d*) treatment may be selected as appropriate when disease progression occurs; and (*e*) the disease may evolve to a more aggressive, and perhaps more "curable" histology.

Unfortunately, data for these hypothetic benefits are very limited. The report by Portlock and Rosenberg (3) retrospectively analyzed the Stanford experience with 44 patients whose treatment was initially deferred. Patients were selected for deferral of initial therapy because they were symptomatically well, had no threatening disease, and were ineligible for protocol study. They were followed closely without initiation of treatment unless disease progression occurred. A recent (August 1981) update of this selected group of patients[4] shows that the median actuarial treatment-free period is 32.5 months for all patients. It is shortest for patients with NML (10 months), intermediate for those with NLPD (55 months), and longest for those with DLWD (73+ months).

Most patients in the Stanford study received "conservative" therapy for disease progression (single-agent chemotherapy, local-field irradiation). The actuarial survival rate remains 60% at 10 years, and there are no significant differences according to histologic subtypes. Moreover, these survival data are not significantly different from results of the ongoing Stanford prospective trials (4). Of the 44 patients in this selected series, six have experienced spontaneous objective regressions, and only two of the six have required the initiation of therapy (after 63 and 110 months of observation) (5). A recent report by Gattiker et al (6) has also documented the frequent occurrence (approximately 8% in their series) of spontaneous objective regressions in nodular lymphoma and their correlation with prolonged survival.

Several groups (7) have reported the development of aggressive histologic subtypes in patients with indolent lymphomas. Its incidence may be as high as 30% in previously treated patients, but its incidence is not known in those who are initially untreated. In previously treated patients, this histologic evolution appears to confer a poor prognosis in spite of intensive therapy. Nevertheless, this prognosis may be related to prior exposure to

[1]Received Oct 13, 1981; accepted Oct 14, 1981.

[2]Section of Medical Oncology, Department of Internal Medicine, Yale University School of Medicine, New Haven, CT.

[3]Nodular lymphocytic, poorly differentiated lymphoma (NLPD); nodular mixed lymphoma (NML); and diffuse lymphocytic, well-differentiated lymphoma (DLWD).

*_Reprint requests to:_ Carol S. Portlock, MD, Section of Medical Oncology, Department of Internal Medicine, Yale University School of Medicine, 333 Cedar St, New Haven, CT 06510.

[4]Horning S, and Rosenberg SA. Personal communication.

chemotherapy rather than to innate drug resistance. An ongoing trial at the National Cancer Institute addresses itself to this question by comparing intensive therapy initiated at diagnosis versus its initiation following disease observation and subsequent progression (8).

Although withholding therapy appears to be an attractive management alternative, it has several potential hazards. First, the patient must receive close and careful followup. There may be silent disease progression in the retroperitoneum leading to marked ureteral deviation and/or obstruction or there may be disease progression into such hazardous sites as the epidural space. Second, some patients may not tolerate the psychologic burden of remaining untreated. Third, once treatment is initiated, its result may be compromised by the advanced state of untreated disease. This remains a theoretic but real concern. It has been reported that systemic symptoms and the presence of extranodal lymphoma involving sites other than bone marrow and/or liver may adversely affect complete response rates and survival (9). Nevertheless, in the retrospective series from Stanford (3) the survival of initially untreated patients was not significantly different from that of the patients who were treated at diagnosis in prospective protocols. Is it possible that the patients whose treatment was initially deferred could have had even better end-results had they received treatment at diagnosis? Finally, is it possible that the evolution to a more aggressive histology while still untreated could diminish survival rather than improve it? Data from previously treated patients (7) and from those with composite lymphoma (10) would suggest that this is possible.

On balance, however, withholding initial therapy in patients with advanced indolent lymphomas who are asymptomatic and do not have threatening disease appears to be a reasonable management option, especially for patients with DLWD and NLPD whose treatment-free period is 55+ months in the Stanford study.[4] Certainly more data regarding the utility and hazards of this approach are needed.

Even more controversial than the question of withholding initial therapy is that of the choice of initial treatment. As emphasized above, virtually all patients whose treatment is initially deferred will require therapy at some time. In the Stanford study[4] the median time to treatment was 32.5 months and histologic subtype was an important parameter. The kinds of systemic therapy which have been studied include daily single alkylating agent, combination chemotherapy, whole-body irradiation, and a combination of chemotherapy and irradiation. To evaluate their relative effectiveness, one must consider the following parameters: (*a*) pathologically documented complete response rate, (*b*) duration of treatment to obtain complete remission, (*c*) durability of complete remission, (*d*) acute toxic effects of therapy, (*e*) chronic toxic effects of therapy, (*f*) ability to salvage following relapse, and (*g*) survival.

In general, complete response rates are not significantly different according to the systemic therapy employed (approximately 60%–80% in most series). The duration of treatment required to obtain a pathologically documented complete response is shortest for whole-body irradiation (6–12 weeks), intermediate for combination chemotherapy (6–12 months), and longest for daily alkylating-agent therapy (12–24 months).

In most studies there do not appear to be significant differences in the durability of complete remissions. Median remission durations in the prospective Stanford studies[5] are approximately 3 years, with a continuous pattern of late relapse (4). Apparent exceptions to these results are the data from the National Cancer Institute. In a group of 31 patients with NML treated with C-MOPP, the complete response rate was 77%, and 79% of complete responders remained in remission at 5 years (11). A recent update (1), however, has reported relapse at 6 years and a pattern of late relapse may soon become evident. Nonetheless, the durability of C-MOPP-induced complete remissions appears superior to that of complete remissions induced by other systemic approaches. A recent prospective study by the Eastern Cooperative Oncology Group (2), where a median disease-free interval of 16.5 months has been reported in 11 complete responders with C-MOPP-treated NML, has not confirmed this conclusion.

The acute toxic effects of therapy are greatest for combination chemotherapy and combined-modality programs and least for daily alkylating-agent therapy. However, in general, the acute toxic effects are manageable and acceptable with most systemic approaches. Data regarding chronic toxic effects or the development of second malignancies are scanty. Combined-modality approaches appear to have the greatest hematologic toxicity, and combination chemotherapy plus whole-body irradiation has been associated with a high risk of acute nonlymphocytic leukemia.

It is not known whether the ability to salvage patients with progressive disease varies according to initial therapy. To date, survival results (60%–80% alive at 5 years) do not suggest a significant

[5]Daily alkylating agent vs combination chemotherapy (CVP) vs combined modality (CVP-total lymphoid irradiation-CVP); daily alkylating agent vs combination chemotherapy (CVP) vs whole-body irradiation.

benefit of any systemic therapy over any other. However, it may require a followup of > 10 years to appreciate any significant differences.

What should be the choice of initial systemic therapy in patients with advanced indolent lymphomas? One must consider all of the parameters discussed above, the patient's disease, and its status. Clearly, the primary goal is to obtain a pathologically documented complete response which is as durable as possible. In this regard, single alkylating-agent therapy appears to be as effective as most combination chemotherapy programs or whole-body irradiation. Whether C-MOPP is significantly better than other systemic therapies in terms of remission durability must await future study. If the patient requires a prompt response to treatment, then single alkylating-agent therapy is inappropriate.

In any patient, one must avoid the pitfall of suppressing disease with continuous treatment rather than attempting to obtain a complete remission. Having deferred the initiation of treatment at diagnosis does not define a "conservative" or "aggressive" course of therapy when the patient's disease progresses. One must choose a systemic approach which fulfills the goal of obtaining a complete remission and avoid unnecessary continuous treatment.

Unfortunately, no curative systemic approach has yet been convincingly demonstrated. Rather than accept this dismal state of affairs, we must continue to search for potentially curative approaches. Just because patients may live with indolent lymphomas for many years does not mean that they will not die from them. We cannot be lulled into calling the indolent lymphomas "favorable" when indeed they are fatal.

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Role of Combined-Modality Therapy in the Curative Management of Advanced-Stage "Favorable" Non-Hodgkin's Lymphomas [1]

Timothy J. Kinsella [2,3,*]

The non-Hodgkin's lymphomas are known not only for their involvement of lymph nodes but also for their ability to spread and involve extralymphatic organs as well. Even so, in the vast majority of patients, lymph node involvement and its secondary manifestations (eg, lymphatic obstruction, bulky mass, hydronephrosis, etc) are the dominant clinical features. This is in sharp contrast to (*a*) patients with carcinomas or sarcomas, in whom the dominant feature is usually primary organ involvement, with secondary involvement of other major viscera, and (*b*) patients with leukemia, in whom the major features (at least initially) reflect the widespread hematologic and hemorrhagic manifestations of that group of diseases. In patients with non-Hodgkin's lymphoma, even those who have bone marrow involvement, hematologic and hemorrhagic manifestations are relatively infrequent, at least at presentation. Thus, for lymphomas of either the Hodgkin's or non-Hodgkin's type, lymph node involvement is the dominant feature in the clinical course of most patients.

The past decade has seen several changes in the management of patients with non-Hodgkin's lymphomas. The development of wide-field, high-dose radiation therapy has been introduced, but with relatively modest gain (1). Certainly the most important development in the management of these diseases has occurred in the area of diffuse histiocytic lymphoma (Rappaport classification), in which combination chemotherapy has unequivocally demonstrated the capability of curing a significant proportion of patients who present with advanced disease (2,3). Although no other development in the previous decade has approached the importance of combination chemotherapy in the management of these diseases, the curability of adult non-Hodgkin's lymphomas of histologic subtypes other than diffuse histiocytic remains generally evasive to such therapy. This is particularly true for the so-called "favorable" histologic subtypes [nodular lymphocytic, poorly differentiated (NLPD); nodular mixed (NM); and diffuse lymphocytic, well-differentiated (DLWD)]. For these histologies, the evidence of curability is generally poor, although there is some suggestion, at least from the National Cancer Institute, that a proportion of patients who have NM lymphoma treated by C-MOPP chemotherapy might be cured by such treatment (4). This has not been confirmed by other investigators (5), and thus for the remainder of this discussion I will take the position that NM lymphoma lacks clear evidence of curability.

The great paradox of non-Hodgkin's lymphoma is that most patients who present with favorable histology tend to have an advanced stage (Ann Arbor stage III or IV) and yet their survival appears to be relatively good (median well in excess of 5 years). Clear understanding of this paradox is lacking, and a much better understanding of the basic biology of these diseases is essential. Precisely because these patients live for prolonged periods, there are major controversies concerning their optimal management.

Currently, there are at least six major ways that such patients are managed: (*a*) initially deferred treatment; (*b*) single-agent chemotherapy; (*c*) combination chemotherapy; (*d*) conventional high-dose radiation therapy (for stage III only); (*e*) whole-body radiation therapy; and (*f*) combined-modality treatment (usually combination chemotherapy and some form of conventional radiation therapy to either all the nodes or at least those of bulky involvement). The thesis of this paper is that combined-modality treatment has the greatest potential for curing advanced non-Hodgkin's lymphoma of favorable subtypes. Toward that thesis, I shall review the results of the various approaches to treatment, with emphasis on curative potential.

The importance of actuarial disease-free data for

[1]Received Nov 13, 1981; accepted Nov 24, 1981.

[2]Radiation Oncology Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

[3]I acknowledge the help and teaching of Drs. Samuel Hellman, George Canellos, and Eli Glatstein during my experience in managing patients with non-Hodgkin's lymphomas.

*_Reprint requests to:_ Timothy J. Kinsella, MD, Radiation Oncology Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, Bldg 10, Rm B3B38, Bethesda, MD 20205.

patients with favorable histologies cannot be overstated. Overall survival data simply provide information concerning the natural history of the disease. Disease-free data plotted as a function of time on an actuarial basis will denote the possibility of a relapse. By definition, a cure will be achieved only when a patient remains free of disease far beyond the statistical likelihood of relapse. To generate these data accurately, careful followup with annual bone marrow evaluation and assessment of the retroperitoneum by repeat lymphangiography and computerized tomographic scanning is necessary. Such followup has rarely been done. It should also be stressed that even 10 years of followup may be insufficient to judge reliably whether or not curative treatment has been delivered.

There has been a recent trend toward recommending no initial treatment in patients with lymphoma of indolent histology, based on a study of 44 selected asymptomatic patients seen over a 15-year period at Stanford University (6). In this "watch-and-wait" approach, close careful followup (every 2–4 months) is required, and treatment is initiated only when there is evidence of disease progression, ie, the development of systemic symptoms or local complications of progressive adenopathy. This approach inherently assumes that the favorable-histology lymphomas are not curable with present-day treatment.

One proposed rationale for a watch-and-wait approach in an asymptomatic favorable-histology patient is that a histologic conversion to a diffuse "unfavorable" histology may occur with disease progression and this patient may then be curable (assuming a conversion to diffuse histiocytic lymphoma). However, in a National Cancer Institute report on 21 patients with histologic conversion, only 15 patients (70%) had a diffuse histiocytic pattern in the lymph node biopsy at relapse, while the other six patients "converted" to diffuse mixed lymphoma (7). Although one third of these patients had a complete response to combination chemotherapy, the median survival of the entire group was significantly shorter than that of relapsed favorable-histology patients who retained their nodular pattern (46 vs 79 months). Only three patients (14%) remain disease-free with a median followup of < 4 years. It is not known whether histologic conversion occurs in favorable-histology patients whose initial therapy is deferred. Additionally, it should be emphasized that up to 20% of patients with non-Hodgkin's lymphoma may have multiple histologic subtypes even at diagnosis, as has been revealed when more than one lymph node is biopsied (8).

Whether combination chemotherapy is superior to single-agent treatment in terms of response rate, actuarial disease-free survival, and overall survival is not clear in favorable histologies. The results of a recent trial in stage III and IV patients at Stanford failed to reveal any response or survival advantage to cyclophosphamide, vincristine, and prednisone (CVP) over single-agent cyclophosphamide or chlorambucil (9). Combining the data from a previous Stanford study (10), the actuarial disease-free data favored single-agent treatment (9). As in most other trials, the complete response rates for combination chemotherapy were quite high ($> 75\%$), but these patients were at continued risk for relapse many years following treatment. This finding also appeared to be true for patients with NM lymphoma treated with C-MOPP therapy and was recently supported in an updated study at the National Cancer Institute (11).

There are definite limitations to the use of conventional radiation therapy alone for advanced favorable lymphomas. First, these patients usually present with involvement (often bulky) of multiple lymph node areas, which requires prolonged treatment and assumes that the patient has good overall health. Second, the possibility exists for further tumor progression in one area not actively treated while a different site is being treated. Third, there is the significant probability of occult tumor beyond the radiation portals such as in other lymph nodes (eg, popliteal or femoral) or especially in the bone marrow. Yet despite these theoretic objections to such treatment, initial relapse in irradiated sites is comparatively uncommon (12), which is the converse of the experience with chemotherapy (13). The survival and freedom-from-relapse data compare favorably to those from other approaches in stage III patients.

The results of high-dose, wide-field total lymphoid irradiation (TLI) in advanced favorable-histology patients are rather scant. If there is any curative potential to primary radiation therapy alone, it would have to be restricted to patients with well-documented stage III disease. At best, this represents a minority of advanced favorable-histology patients. The radiation technique for these patients must be distinguished carefully from the techniques used for the treatment of Hodgkin's disease. A wide-field approach to the abdomen appears essential if radiation therapy alone is used, because of the high propensity for mesenteric nodal involvement even at presentation. Conversely, the infrequent involvement of the mediastinum at presentation or relapse argues for an alteration of the supradiaphragmatic radiation technique ("mini-mantle") to limit lung and heart irradiation in this typically middle-aged group of patients.

The results of TLI in patients with stage III nod-

ular lymphoma are quite provocative. In a group of 51 stage III patients treated at Stanford, the actuarial relapse-free survival was 35% at 10 years (1). A smaller series from another institution achieved even better results, with 58% disease-free survival in patients with stage III nodular lymphoma at 10 years (14), although it should be restated that 10-year actuarial disease-free survival might be insufficient to judge reliably whether or not curative treatment has been delivered. For patients with indolent lymphoma, 15 years of followup may be necessary for the possibility of having achieved a "cure" to be entertained.

The use of total-body irradiation as a systemic treatment offers some theoretic advantage over TLI. The response rates and overall survival are comparable to those achieved by either single-agent or combination chemotherapy (9,15). However, long-term followup shows that virtually all of these patients have relapsed (16). Total-body irradiation should be considered a palliative modality with no evidence of curative potential when used either alone (15,16) or in combination with chemotherapy (9).

Given the rather modest disease-free results for stage III patients receiving radiation therapy, and at least a potential curative role for combination chemotherapy, it is surprising how seldom the two modalities have been combined to treat patients with advanced favorable lymphoma. A combined-modality approach appears attractive when the patterns of failure are analyzed in patients with favorable lymphoma treated with either radiation therapy or combination chemotherapy. With careful followup of stage I, II, and III patients treated by radiation therapy alone, long-term local control of involved lymph nodes varied from 92% to 98% in one study (12) (table 1). Two other interesting findings in the followup of these patients were: (*a*) among patients relapsing in nodal sites, only one half relapsed in contiguous nodal areas which might have been included in wide-field irradiation; and (*b*) approximately one third of relapsing patients failed in extranodal sites with or without lymph node failure. Both findings underscore the limitations of conventional high-dose irradiation alone in managing patients with advanced favorable lymphoma. Additionally, since each site of lymph node involvement appears independent of other lymph node areas in terms of local control, it is not surprising that the actuarial disease-free survival for a heterogeneous group of stage III patients is only 35%.

The typical stage III patient has involvement of multiple lymph node areas on both sides of the diaphragm. The theoretic probability of tumor control or "cure" (Pc) is a function of local control within each involved lymph node site. Thus, $Pc = (1 - R)^n$, where R = recurrence rate for a single involved site and n = number of sites involved. Assuming a recurrence rate of 10% for a lymph node site treated with > 3500 rad (12) and five or six involved lymph node sites in a stage III patient, the probability of "cure" is 30%–39%, which is remarkably consistent with the long-term disease-free data from Stanford (1).

With combination chemotherapy, relapse in previously involved lymph nodes is a more common problem (table 2). The best group appears to be patients with NM histology, where local control in lymph nodes was established in only 65% of the 31 patients. It can be assumed that those patients who did not achieve complete remission had persistent nodal disease; 80% of relapses occurred in nodes only (4). Thus, in over one half of the patients with favorable-histology lymphoma, lymph nodes were *not* controlled. With longer followup, a further drop in the disease-free survival curve is reported, presumably with more nodal relapses (11). Local lymphatic control in patients with NLPD or DLWD histology was only 25%.

Combined-modality therapy with high-dose TLI and CVP chemotherapy has been used in a prospective trial in patients with stage IV nodular lymphoma at Stanford (17). The TLI was given after two cycles of CVP chemotherapy and then patients received additional cycles of CVP. The complete response rate, median survival, and actuarial disease-free survival did not differ from those of patients who were randomized to receive CVP alone or even single-agent chemotherapy. Although this study failed to show any advantage to combined-modality therapy, there was a major flaw in its design which may explain the results. The study was limited to stage IV patients, virtually all of whom had bone marrow involvement. Following two cycles

TABLE 1.—Local control of lymph node involvement in patients with favorable lymphoma treated with radiotherapy (≥ 3500 rad)*

Histology	Stage			Total No. of patients	No. of failures in irradiated nodes	% local control of nodal disease
	I	II	III			
NLPD	11	16	22	49	1	98
NM	9	16	31	56	3	92

*From ref 12.

TABLE 2.—Local control of lymph node involvement in patients with advanced favorable lymphoma treated with CVP, C-MOPP, or BACOP*

Histology	No. of patients	No. of complete remissions	No. not relapsed	No. of nodal relapses†	Total No. of nodal failures
NLPD	49	33	12	17	33 (67%)
NM	31	24	20	3	10 (32%)
DLWD	11	7	2	4	8 (73%)
Total	91	64 (70%)	34 (53%)	24 (27%)	51 (56%)

*From ref 4.
†80% of relapses occurred in lymph nodes only.

of CVP, the bone marrow was not rebiopsied to assess response and clearance. Instead, patients received TLI for approximately 4 months prior to reinstitution of systemic therapy. One questions whether better results might have been found if a pathologic complete response was required of the chemotherapy prior to the use of TLI. The rationale for this approach is predicated on the observation of a continuous relapse rate following a complete response to combination chemotherapy (13).

The use of "consolidation" irradiation following a complete response to combination chemotherapy might be considered as the ultimate in "non-cross-resistance" based on the pattern of lymph node relapse when combination chemotherapy or radiation therapy is used alone. A major issue in a combined-modality approach is how combination chemotherapy and irradiation might be integrated to optimize curative potential and minimize morbidity, especially hematologic toxicity. Following clinical and pathologic staging (usually without laparotomy), patients could begin to receive combination chemotherapy until a complete clinical response has resulted. If the complete response is confirmed by pathologic restaging (bone marrow biopsies, peritoneoscopy, etc), another two to three cycles of chemotherapy might then be given prior to initiation of radiation therapy. Another approach after a pathologic complete response would be the repetitive alternation of several cycles of chemotherapy with large fields of irradiation as described for advanced Hodgkin's disease (18).

The dose and volume of irradiation are two additional variables to be considered in the optimal design of a combined-modality regimen. Proper radiation technique entails the use of large irradiation fields which encompass multiple adjacent lymph node areas and yet shield normal tissues. The use of equally weighted, opposed fields allows for improvement of dose homogeneity. The use of a simulator, computerized tomographic scanner, and contoured blocks is of utmost importance. Since most patients will have involvement of multiple lymph node areas above and below the diaphragm, wide-field irradiation to these lymph node sites seems most reasonable. However, with such extensive irradiation, the tolerance of the heart, lung, kidney, bowel, and liver, in addition to bone marrow, must be kept in mind. As previously mentioned, the infrequent involvement of the mediastinum and hila at presentation and relapse in these favorable-histology patients allows for a modification of supradiaphragmatic irradiation (mini-mantle) to limit heart and lung irradiation. This is especially important if doxorubicin and bleomycin are used. On the other hand, the high probability of mesenteric lymph node involvement demands whole-abdominal pelvic irradiation which will include the liver, kidney, and bowel, as well as the lumbar vertebral and pelvic bone marrow. A wide-field abdominal technique employing both opposed anteroposterior-posteroanterior fields and opposed lateral fields is recommended (19). The total dose of irradiation required to establish long-term control in lymph nodes after a complete response to combination chemotherapy is not known. When radiation therapy is used alone, it appears that a total dose as low as 2500 rad may be sufficient to accomplish this aim (14). In a combined-modality approach, TLI to 2000–2500 rad after a complete response to chemotherapy should be associated with relatively minimal morbidity.

The potential leukemogenic consequence of combined-modality therapy in these favorable-histology patients is of concern in light of the evolving data on combined-modality therapy for Hodgkin's disease (20). However, it should be noted that the chemotherapy usually associated with this uncommon but dreaded late complication includes procarbazine, a drug known for its potential leukemogenicity in animal studies (21). Procarbazine is not commonly employed in the treatment of the favorable-histology patients.

In summary, a combined-modality approach using combination chemotherapy and high-dose, wide-field irradiation appears to have the greatest curative potential for patients with advanced non-Hodgkin's lymphomas of the favorable histologic subtypes. The major argument supporting this ap-

proach stems from an analysis of the patterns of relapse following either combination chemotherapy or radiation therapy alone. Since most patients have stage IV disease at presentation, emphasis should be placed on documenting a pathologic complete response to the initial chemotherapy (especially in bone marrow) prior to wide-field irradiation. In this setting, long-term control ("cure") of lymph node disease appears possible.

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Streptozocin: A Review of Its Pharmacology, Efficacy, and Toxicity[1]

Raymond B. Weiss[2,3,*]

Streptozocin (STZ) is a glucosamine-nitrosourea compound that has been in clinical trial since 1967. It was formerly designated streptozotocin, but its name has been shortened by the US Adopted Names Council. A New Drug Application has been filed with the US Food and Drug Administration by The Upjohn Co (Kalamazoo, MI) for marketing under the trade name Zanosar. Approval of this application is imminent. In anticipation of marketing, the chemistry, pharmacokinetics, clinical efficacy, and toxicology of this drug will be reviewed.

HISTORICAL BACKGROUND

While searching for new antimicrobial agents produced by *Streptomyces* organisms derived from soil samples, investigators at the Upjohn Research Laboratories isolated a new antibiotic in 1956 (1). The soil sample had been randomly collected by an Upjohn product salesman near Blue Rapids, KS. The new substance was derived from a fermentation broth of *Streptomyces achromogenes*, variety 128, a brown pigmented variety. It was named streptozotocin to take into account its *Streptomyces* origin and its azo, or nitrogen-containing, chemical structure. STZ was found to have a significant antimicrobial action for a wide spectrum of organisms (1,2).

In 1960 and 1961, antitumor activity screening at both Upjohn Research Laboratories and contract laboratories of the National Cancer Institute demonstrated antitumor effect, particularly in L1210 leukemia (3). These results created interest in the drug as an antitumor agent. During the preclinical toxicology studies, it became apparent that when the drug was administered iv at a sufficient dose, it produced hyperglycemia in rats and dogs within a few hours of treatment (4,5). Thus, despite good antimicrobial activity, the drug was too toxic to be used as an antimicrobial agent. Preclinical toxicology studies were completed in the mid-1960s, and clinical trials in cancer patients were begun in 1967.

CHEMISTRY AND FORMULATION

STZ was originally derived from a fermentation process, but its structural simplicity and the high production cost of fermentation spurred successful efforts to synthesize it. The structure (fig 1) is composed of a nitrosourea moiety with a methyl group attached at one end and a glucose molecule at the other. The molecular weight is approximately 265.

The drug is prepared in 1-g vials as a crystalline powder that is very water-soluble. The vials also contain 120 mg of anhydrous citric acid to provide buffering at an acidic pH and to insure stability. A solution is made with sterile water which can be added to either 5% dextrose or saline for administration without loss of potency or stability. Unopened vials should be stored in the refrigerator. Once reconstituted, the solution is stable for 24 hours under refrigeration.

PRECLINICAL EVALUATION

Antitumor effect against L1210 and P388 leukemias was demonstrated at various doses and with both sc and ip injection (3,5,6). Oral administration required four to six times the parenteral dose to produce the same effect (3). STZ was also active in Ehrlich ascites carcinoma and Walker 256 carcinosarcoma (5), and there was minor activity in Sarcoma 180. It was inactive in Lewis lung carcinoma and Carcinoma 755 (3,5).

Murine tumor studies led to the discovery that STZ produced hyperglycemia (4,5); further toxicology studies in dogs and rhesus monkeys demonstrated that STZ had a potent diabetogenic effect (4,7). It could produce permanent diabetes with its full manifestations including ketoacidosis. Histologic studies of pancreatic tissue in animals showed rapid degranulation of islet beta cells without necrosis (4,8). Subsequent studies by Junod et al (9) showed that the diabetogenic effect was maintained through a range of doses. The induction of diabetes in certain animals is so uniform that STZ is widely

[1]Received Apr 23, 1981; revised Sept 28, 1981; accepted Oct 7, 1981.

[2]Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute; and Uniformed Services University of the Health Sciences, Bethesda, MD.

[3]I am grateful to Dr. Gary L. Neil of The Upjohn Co for his invaluable assistance in the preparation of this paper.

*Reprint requests to: Raymond B. Weiss, MD, Section of Medical Oncology, Walter Reed Army Medical Center, Washington, DC 20012.

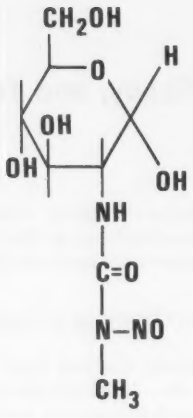

FIGURE 1.—Structure of STZ.

used to produce an experimental model for the study of diabetes. However, the diabetogenic effect is absent in guinea pigs and only occurs in rabbits at very high doses (10). This species variability is carried through to man, because STZ does not produce any significant clinical diabetogenic effect.

Other STZ toxicity in dogs and monkeys included hepatotoxicity manifested by elevated serum hepatic enzymes without hyperbilirubinemia (7). Interstitial nephritis was also noted without azotemia (7).

MECHANISM OF ACTION

STZ undergoes spontaneous decomposition to produce reactive methylcarbonium ions that alkylate DNA and cause interstrand cross-links (11). However, in comparison to other nitrosoureas, its alkylating activity is weak (12). The methylnitrosourea metabolite has three to four times the alkylating activity of the parent compound, so the presence of the sugar moiety reduces the alkylating action (13). The glucose moiety also reduces the bone marrow toxicity, because methylnitrosourea has several times the myelosuppressive effect of the parent compound (13). STZ is also known to inhibit precursor incorporation into DNA of mammalian cells (14,15). It has a lesser effect on RNA and protein synthesis (6). It is phase-nonspecific and can affect cells in all stages of the life cycle, but it particularly blocks progression from G_2 to M phase (14).

STZ also has the ability to carbamoylate lysine residues of nucleoproteins after formation of an isocyanate. Again, in comparison to the other nitrosoureas, STZ also has low carbamoylating activity (12). The attachment of the methylnitrosourea group to the glucose moiety at the C2 position results in this low carbamoylating activity (15). Studies by Panasci et al (13) have shown that the antitumor efficacy of STZ does not correlate with the degree of carbamoylating action, so carbamoylation appears to play little or no part in tumor cytotoxicity.

The diabetogenic action of STZ in animals is mediated through a reduction of nicotinamide adenine dinucleotide (NAD) in pancreatic cells (16,17). The NAD decrease appears to be due to a combination of reduced cell uptake of precursors and reduced NAD synthesis within the cell (18). The diabetes can be prevented, with no interference in the antitumor effect, by the administration of nicotinamide (16,17).

Animal studies of the pancreatic islet cell uptake of STZ and its metabolite methylnitrosourea demonstrate that the sugar moiety is important for such uptake to occur. Anderson and colleagues (19) showed that at equimolar doses of methylnitrosourea and STZ, approximately four times greater uptake of STZ than the nitrosourea occurred in the islet cells. Also, 3.5 times more nitrosourea than STZ was required to produce diabetes.

It is apparent from the above biochemical studies that the nitrosourea-glucosamine complex is important in the drug's biologic effect. The nitrosourea moiety is necessary for DNA alkylation and antitumor efficacy, and the sugar moiety is necessary to facilitate islet tissue uptake and minimize bone marrow toxicity.

PHARMACOKINETICS

Parent compound cannot be detected in the plasma beyond 3 hours after administration (20). The initial half-life is 5–15 minutes, and the terminal half-life is 35 minutes (20,21). Metabolites can be detected in plasma up to 24 hours after administration. There is little or no parent drug transport into cerebrospinal fluid. However, STZ metabolites do readily enter the spinal fluid, and 2 hours after administration they are present at a concentration nearly the same as that in plasma (20).

Tissue distribution studies in animals have identified the liver and kidney as the organs with highest drug concentration (21). By 1 hour after iv administration in mice, only 10 μg/ml of STZ was present in the plasma, but 90 μg/g was present in the liver (21). Whereas the mouse and dog had higher drug concentrations in the liver than in the kidney, the monkey had approximately 2.5 times greater drug concentration in the kidney than in the liver (21). Drug also concentrated to some degree in the pancreas and intestine. A significant concentration of

drug was detected in the lungs of monkeys (21). No drug concentrated in the brain.

STZ is rapidly eliminated by the kidney, and the high renal concentration of drug reflects this urinary excretion. In human studies, 60%–72% of the administered dose can be detected in the urine within 4 hours of administration (20–22). Parent drug accounts for only 10%–20% of the excretion; the rest is metabolites. Most excretion takes place by 24 hours.

ADMINISTRATION AND DOSE

STZ is usually administered iv. An oral preparation is not available because in the early antitumor studies in animals, four to six times more oral drug was required to produce the same effect achieved iv (3). Also, no investigations of absorption and bioavailability have been done.

Some investigators have successfully used STZ in an intra-arterial infusion. Kahn et al (23) administered STZ into the hepatic arteries of two patients with liver metastases of islet cell tumors. Both patients had good objective biochemical and tumor responses, and there was no hepatic drug toxicity. Other investigators have also safely used this route in a few patients (24). The safety of this route of administration has encouraged the Southwest Oncology Group (SWOG) to use it in a study of STZ infusion therapy for hepatoma.

The two most widely tested dose schedules are 1.0–1.5 g/m^2 given at weekly intervals (24) and 500 mg/m^2 given daily for 5 days every 6 weeks (25). Both schedules are safe and tolerable. Neither has an advantage in toxicity, but the intense vomiting induced by STZ may be more acceptable to the patient when it occurs only every 6 weeks instead of weekly.

CLINICAL EFFICACY

Islet Cell Tumors

The murine tumor studies revealed that STZ had a clear hyperglycemic effect. Thus, in the initial clinical trials in 1967, interest was focused on patients with pancreatic islet cell tumors. Murray-Lyon and colleagues (26) were the first to use STZ in the treatment of an insulin-producing metastatic islet cell tumor. This patient had an objective decrease in both measurable tumor and hormone production. Since this initial case, there have been numerous reports documenting STZ as an effective drug in this rare tumor (24,27–29).

The series of 52 patients collected by Broder and Carter (24) through the National Cancer Institute is the largest number of patients reported. Among the cases evaluable for response, 54% had reductions in biochemical manifestations of the tumors and 37% had at least partial tumor responses. Those patients with hypoglycemia, who had a serum insulin decrease as a result of treatment, improved dramatically. The patients achieving objective response had a significant increase in survival over the nonresponders at 1 year (24). Responses also occurred in patients with tumors that secreted proinsulin.

Both beta and non-beta cell islet tumors that secrete a variety of other polypeptide hormones are also responsive to STZ. STZ has effected responses in such tumors producing the following hormones: gastrin (26,30–33), adrenocorticotropin (34), a parathormone-like substance (35,36), somatostatin (37), calcitonin (36), alpha subunit of chorionic gonadotropin (38), glucagon (26,39–41), serotonin (28), vasoactive intestinal peptide (42–47), and undefined hormones sometimes causing the watery diarrhea syndrome (23,48). Both biochemical and tumor responses have occurred with all these islet cell tumors. When objective responses occurred, symptoms as a result of the tumor and its hormone secretion decreased to minimal levels or disappeared.

STZ is equally effective in nonfunctioning islet cell tumors. Moertel et al (25) noted an identical response rate (36%) to STZ, whether the tumor was hormonally functional or not.

5-FU is occasionally active in islet cell tumors (24,48). The lack of overlapping toxicity between 5-FU and STZ, and their individual activity, suggested that combining them would be useful. Individual patients treated with this combination have had striking remissions (49–51). In a pilot study by Moertel (48), objective responses occurred in six of eight patients. This experience formed the basis of a large randomized trial by the Eastern Cooperative Oncology Group (ECOG) comparing STZ alone with STZ plus 5-FU (25). Both drugs were administered for 5 days every 6 weeks; 84 patients were evaluable. The combination regimen produced both a greater overall rate of response (63% vs 36%) and greater rate of complete response (33% vs 12%). The median survival with the combination was also greater (26 vs 16.5 months), but this difference did not reach statistical significance. Responses occurred in tumors producing all the various hormones, and there was no preferential sensitivity of any of the functional types. The response rate of the drug combination was higher in the functional tumors than in the nonfunctional ones (80% vs 62%), but this difference was not statistically significant. The drug combination was more toxic to the bone

marrow, but this was life-threatening for only a small minority of patients. One death occurred as a result of leukopenia and sepsis. The ECOG has now embarked on a randomized comparison of STZ plus 5-FU versus STZ plus doxorubicin. A third arm in this trial is chlorozotocin, to study the efficacy of another nitrosourea with a glucose moiety in the treatment of this disease. Patients failing treatment with one nitrosourea are treated with the other to determine any cross-resistance.

Carcinoid Tumors

A number of cases of carcinoid tumors with objective and/or biochemical responses to STZ have been reported (52–55). It thus appears that this drug's striking activity in islet cell tumors extends to other tumors derived from amine precursor uptake and decarboxylation (APUD) tissues.

5-FU is also active in carcinoid tumors and produced objective responses in six of 15 patients in a Mayo Clinic study (48). A combination of STZ and 5-FU was well-tolerated and more active in islet cell tumors, so a pilot study of the combination was also tried in carcinoid tumors. Six of nine patients had responses (48). Other investigators were also able to achieve objective tumor and biochemical responses with this combination (56).

These promising results led to an ECOG randomized trial comparing STZ plus 5-FU versus STZ plus cyclophosphamide (57). Cyclophosphamide was also considered to be an active drug in the treatment of carcinoid tumors. Among 89 evaluable patients, the 5-FU combination induced a 33% response rate and the cyclophosphamide combination induced a 26% response rate; this was not a statistically significant difference (57). The response rates were better for the subgroups who had tumors of small bowel origin (up to 44% for the 5-FU combination). The median response duration was 7 months. There was more toxicity from the cyclophosphamide combination, suggesting that the STZ and 5-FU combination is preferable. This study did not address the question of whether STZ plus 5-FU is better than STZ alone.

Doxorubicin is another active drug in carcinoid tumors (58). SWOG has initiated a trial using the four-drug combination of STZ, 5-FU, doxorubicin, and cyclophosphamide in an attempt to increase the response rate of the STZ plus 5-FU combination.

Other APUD Tumors

STZ has been tried in a few other metastatic APUD cancers, but so far without success. Hamilton et al (59) treated two patients with pheochromocytomas and neither responded, either biochemically or with tumor regression. Weiss (60) and Norton et al (61) treated two patients with calcitonin-producing medullary carcinomas of the thyroid and neither patient responded. Weiss (60) also treated a patient with an ACTH-producing malignant thymoma, and again there was no biochemical or tumor response to STZ.

Hodgkin's Disease

The value of STZ as part of "second-line" chemotherapy for Hodgkin's disease is as yet undefined. Schein and colleagues (28) observed seven objective responses, including one complete response, among 16 previously treated patients. Four of these patients were previously refractory to the nitrosourea carmustine (BCNU).

Approximately 80% of patients with advanced Hodgkin's disease have a complete remission induced by one of several four-drug regimens such as MOPP. For the other 20% who fail to respond and for those who relapse after MOPP treatment, alternative non-cross-resistant regimens must be developed. Such a regimen, consisting of STZ, lomustine (CCNU), doxorubicin, and bleomycin, was devised by Levi et al (62). This combination was initially used in previously treated patients and produced a 35% complete, and 59% overall, response rate. Toxic effects of several kinds were major problems with this regimen.

The promising results of this STZ-containing treatment encouraged these investigators to use it in untreated patients with advanced disease (63). In this more favorable group the regimen produced complete remissions in 15 of 20 (75%) patients. Since it was effective as initial chemotherapy, this regimen plus MOPP is being tested in an alternating schedule against MOPP alone (64). It is hoped that this eight-drug sequence will be more effective, outweighing the greater toxicity from such a regimen.

Vinciguerra and colleagues (65) conducted similar studies in previously treated patients. Using a combination of bleomycin, vinblastine, doxorubicin, and STZ, they had five responses in ten patients who were resistant to MOPP. These investigators incorporated this combination into a cooperative group study comparing alternating eight-drug therapy with two different four-drug combinations. The alternating regimen was slightly better than either four-drug combination, but the difference was not significant (66).

Although it appears that non-cross-resistant regimens containing STZ produce useful responses in Hodgkin's disease, the contribution of STZ to achieving these responses is unclear, and no study has attempted to isolate its effect.

Colon Carcinoma

As with Hodgkin's disease, it is not yet clear what benefit, if any, STZ has in colon cancer.

5-FU has been the standard agent for the treatment of metastatic disease for > 20 years. Unfortunately, the objective response rate is only about 20%. Prodigious effort has been expended in screening new drugs, including STZ, for activity in this disease. Horton et al (67) tested STZ in 50 previously treated patients, and five (10%) had partial remissions. Douglass et al (68) had five (15%) partial responders among 33 previously treated patients. This level of response was not enough to excite interest in further trials with STZ in this disease. However, a group at Sloan-Kettering Memorial Hospital recently added STZ to the three-drug combination of methyl-CCNU, vincristine, and 5-FU, building on their previous experience using these three drugs together (69,70). The acronym for this four-drug regimen was "MOF-Strep." Among 77 previously untreated patients, 24 (32%) had remissions from MOF-Strep (70). The median survival of responders was double that of nonresponders (14 vs 7 months). Their previous trial of the three-drug combination in 67 patients demonstrated only an 11% response rate (69). These results suggest that STZ added something to the therapeutic effect.

These data from a single institution need confirmation, and the Gastrointestinal Tumor Study Group has begun a trial using the same schedule of MOF-Strep in patients with no prior chemotherapy. It is hoped that this STZ combination, if active, can then be used as adjuvant therapy. The North Central Cancer Treatment Study Group is doing similar work comparing MOF-Strep to 5-FU alone in advanced disease.

Pancreatic Ductal Adenocarcinoma

Although STZ has clear efficacy in pancreatic islet cell tumors, it appears to have minor effect in pancreatic ductal cancers. Two isolated responses to STZ in nine patients with pancreatic adenocarcinoma have been reported (54,71), but they were brief. Carter and Comis (72) reported three responses in 27 patients. Moertel used STZ plus 5-FU in four patients and two responded (48). Seligman et al (73) used the same combination in 19 patients, and four responded.

With these data as background, three studies of STZ combination therapy in this tumor were begun. Two were conducted by the ECOG (74,75) and one was conducted by the Georgetown University group (76).

The first ECOG study tested STZ plus 5-FU versus STZ plus cyclophosphamide in 93 evaluable patients (74). Each combination had only a 12% response rate, and there was no significant difference in survival between the two regimens. A subsequent study compared 5-FU alone with STZ plus 5-FU in 176 patients (75). Objective response data were not given, but there was no survival difference between the two treatments (median: 16 vs 18 weeks).

The Georgetown combination was STZ, mitomycin, and 5-FU (SMF) (76). Ten (43%) of 23 untreated patients responded, which is strikingly better than noted in the ECOG trials. Another group, using the same regimen with slightly lower drug doses, achieved responses in seven of 22 patients (77).

These preliminary results encouraged SWOG to conduct a randomized trial comparing SMF to mitomycin plus 5-FU. A recent preliminary communication on data from 95 patients relates a response rate of 40% for SMF versus a response rate of 5% for the two-drug combination (78). There was, however, no significant difference in median survival between the two regimens. Other cooperative groups such as the Cancer and Leukemia Group B and the Gastrointestinal Tumor Study Group are also testing SMF, but no results are yet available.

With the exception of the limited experience with SMF, STZ has not shown much benefit whether it is used alone or in combination. Although the early SMF studies are promising, they need to be confirmed.

Miscellaneous Cancers

STZ has been tested in a variety of additional cancers, but in most there have been few or no responses.

Falkson and co-workers (79) did a randomized trial comparing four treatments of hepatoma, two of which were oral 5-FU plus STZ and oral 5-FU alone. None of 43 patients in the 5-FU-alone group responded; four (12%) of 33 patients in the two-drug group had remissions (79).

These data suggest that STZ has some minor activity in hepatoma. Ihde et al (80) attempted to improve the response rate by using a combination of doxorubicin plus STZ in a small series of patients, but they were not able to achieve a rate better than 15%.

It appears that STZ is only weakly active in hepatoma, but SWOG is attempting to do better in an ongoing study by administering 5-FU, doxorubicin, and STZ together in a hepatic artery infusion. Those patients achieving at least a partial remission then receive the same drugs iv on a maintenance sched-

ule. This study, however, will not demonstrate whether STZ is a necessary part of such treatment.

Since STZ was active in L1210 leukemia (3), it was tested clinically in acute lymphatic leukemia. It had no activity in two series with adequate numbers of patients (81,82), and further studies have been abandoned.

The activity of STZ in some APUD cancers encouraged examination of its effect in small cell carcinoma of the lung, another hormone-producing APUD tumor. In four studies comprising a total of 56 patients (83–86), there were no responses. Thus, small cell carcinoma is decidedly unresponsive to STZ.

STZ has been studied in breast cancer in only one reported trial (87). There were no responses among 20 heavily pretreated patients.

Because of its lack of myelosuppressive toxicity, STZ was incorporated into a combination regimen that also included bleomycin and methotrexate to treat epidermoid carcinoma of the lung (88). Despite a response rate of 38% in 16 patients, the investigators encountered too much toxicity to pursue this regimen further.

The Veterans Administration Lung Cancer Study Group compared STZ with a variety of other drugs for extensive-stage, non-small cell lung cancer (84). The data were not reported as response rates, but STZ appeared to be an ineffective drug for this disease.

The National Prostatic Cancer Project used STZ as a nonmyelosuppressive agent for patients who had previously had pelvic irradiation (89). Among 38 patients, there were 12 whose disease stabilized, but no responses occurred. Estramustine was a more promising agent in this study, and further trials with STZ were abandoned.

After noting several responses to STZ alone in a pilot study, Chang and Wiernik (90) did a randomized comparison of doxorubicin alone versus doxorubicin plus STZ in metastatic sarcomas. Unfortunately, the response rates for the two treatments were the same, while the toxicity of the STZ combination was much greater. No other studies of STZ in sarcoma have been attempted.

TOXICITY

Renal

Renal dysfunction is the major dose-limiting toxic effect of STZ and occurs in 28%–73% of patients. In the first phase I trial, all 18 patients developed some form of renal dysfunction and two became anuric (91). Schein et al (28) noted renal abnormalities in 28% of 106 patients treated with STZ. Nephrotoxicity was the most common side effect and contributed to the death of four patients (28). Moertel and co-workers (52) saw evidence of STZ nephrotoxicity in two thirds of 38 patients. Broder and Carter (24) also noted that two thirds of the patients in their series had renal toxic effects, and five died. Stolinsky et al (54) observed one or more manifestations of STZ nephrotoxicity in 55% of 51 patients. The incidence of renal toxicity rose with prolonged drug administration in all these series.

The clinical manifestations of STZ nephrotoxicity involve abnormalities of both glomerular and tubular function. Sadoff (91) detected hypophosphatemia as the earliest sign of toxicity, and this appeared in some patients after just one drug dose. Glycosuria, acetonuria, hyperchloremia, and aminoaciduria were also seen (91), indicating renal tubular acidosis. Schein et al (28) also noted a high frequency of renal tubular acidosis. Broder and Carter (24) reported that proteinuria occurred in one half of 52 patients and appeared in some patients after only a few drug doses. Other investigators (28,52,71) also have found proteinuria to be common, occurring in up to 73% of patients. Protein excretion levels are usually low (1.0 g/24 hours), and only rarely have they approached nephrotic syndrome levels (> 3.0 g/24 hours) (52). Elevations in BUN and serum creatinine are later findings and sometimes do not occur despite the presence of other renal abnormalities (28).

If the indicators of nephrotoxicity are only mildly abnormal and the drug is stopped, they may return to normal. However, with continued treatment, irreversible (and sometimes fatal) renal toxic reactions will occur in most patients.

The sites of STZ renal injury are both the glomerulus and tubule, because histologic changes have been observed in both structures (24,36,92,93). Myerowitz et al (36) noted extensive tubular atrophy and interstitial inflammatory infiltrates plus glomerular cellular tufting in the renal tissues of an intensively studied patient. In another patient similarly studied, histologic changes were most prominent in the proximal convoluted tubules and collecting ducts (93). Fennell and Falls (94) studied the renal physiology of a patient with renal tubular acidosis secondary to STZ nephropathy and localized the urine acidification defect to the proximal tubule.

As previously discussed, the major excretion pathway of STZ and its metabolites is the kidney. However, there is no explanation at the cellular and molecular level for glomerular and proximal tubular sensitivity to STZ. Exploration of the mechanism could possibly point to methods for limiting or preventing nephrotoxicity, thus providing a better therapeutic index.

Vomiting

Vomiting occurs in > 90% of patients receiving STZ, beginning 1–4 hours after administration. For about 10% of patients the vomiting can be severe and protracted (24,28). It is rarely lessened by phenothiazines, but newer antiemetics such as tetrahydrocannabis and metoclopramide may be more efficacious.

Vomiting is the most noxious side effect for patients and frequently causes them to refuse further STZ treatment. Exploration of means of reducing emesis would be particularly useful for this drug. One lead in this regard was provided by a phase I study of STZ administered by a 5-day continuous infusion (95). Of 19 patients so treated, six had no nausea or vomiting, and seven had only nausea with no vomiting (95). These results, if confirmed and if antitumor efficacy is maintained, suggest that there are effective ways of reducing this unpleasant toxic effect of STZ.

STZ is rapidly metabolized, and it is presumed that the vomiting is caused by action of one or more metabolites on the brain stem vomiting centers. However, this subject has never been investigated, and it is not known how STZ-induced emesis is mediated.

Bone Marrow

STZ usually has little hematologic effect, which makes it a useful drug in combination with other drugs that do cause myelosuppression. Leukopenia and/or thrombocytopenia occurred in only 9% of 106 patients in one series, with the nadir being 1–2 weeks after treatment (28). In 52 patients, only 20% had hematologic changes, and most of these patients had mild anemia. However, a few cases of leukopenia, sepsis, and death have occurred (24,28). Four patients have had asymptomatic eosinophilia, apparently related to STZ, that disappeared after drug withdrawal (28,54,95).

Chang et al (96) found inordinate leukopenia and thrombocytopenia when they used STZ in combination with doxorubicin compared with use of doxorubicin alone. The dose of doxorubicin used with STZ was 60 mg/m^2. Pharmacokinetic studies of patients receiving the two drugs demonstrated a significantly prolonged excretory half-life of doxorubicin (96). The reason for this observation may be that STZ inhibits hepatic elimination of doxorubicin. If these two drugs are used in combination, the doxorubicin dose must be reduced from the level usually used as single-agent therapy.

It appears that STZ and a myelosuppressive nitrosourea can also synergistically enhance hematologic effect. When STZ and carmustine (BCNU) were given together, Lokich et al (97) noted much lower nadir blood cell counts (particularly platelets) than would be expected from BCNU alone. How this apparent synergism is mediated is not known.

Glucose Intolerance

STZ produces diabetes in some animals, but not in others (4,5,10). It causes no more than mild, inconsequential glucose intolerance in man. In one study (54), six patients had mild serum glucose elevations from STZ, but in none did the fasting glucose exceed 134 mg/dl. Sadoff (98) measured serum glucose in 15 patients and found only mild glucose intolerance in nine. None required insulin. Some of these patients (98) had glycosuria without hyperglycemia, presumably due to the effect of STZ on the proximal renal tubules. Only 6% of patients in another series had impaired glucose tolerance, and none required treatment (24). Even in patients who initially have had abnormal glucose tolerance due to their cancer, this effect was not exacerbated by STZ treatment (99).

Hepatic

Chemical liver dysfunction occurs in approximately 25% of patients receiving STZ, but only occasionally is it severe (24,25,28,98). Elevated serum enzymes appear 1–2 days after a drug course and rapidly return to normal (28). Two patients with chronic forms of liver toxicity have been noted, but these patients also received BCNU, so the abnormalities possibly were not due solely to STZ (100).

Miscellaneous Toxic Effects

Figure 2 lists the miscellaneous and uncommon side effects that have been reported from STZ. No pulmonary toxicity has been seen, although it is well-known that other nitrosoureas have caused such an effect (101). Cardiac arrhythmia was recorded in two patients receiving STZ (24), but there have been no other such cardiotoxic effects. No instances of overt hypersensitivity reactions have occurred, although the previously mentioned eosinophilia may be a subclinical form of such allergic phenomena. Severe skin necrosis in the hand occurred in one patient who had sc drug infiltration (102), so care must be taken during drug administration to prevent this problem.

The only instances of central nervous system toxicity reported were in a study of continuous STZ infusion over 5 days (95). Five of 19 patients developed confusion, lethargy, and depression. This

MAJOR

1. Nephrotoxicity: Proteinuria
   Glycosuria
   Azotemia
   Hypophosphatemia
   Hypokalemia
   Hypocalcemia
   Hyperchloremia
2. Nausea and Vomiting

MILD TO MODERATE (ONLY RARELY LIFE-THREATENING)

1. Myelosuppression: Anemia
   Leucopenia
   Thrombocytopenia
2. Hepatotoxicity: Elevated serum enzymes
   Hyperbilirubinemia

MINOR and/or RARE

1. Eosinophilia
2. Glucose Intolerance
3. Vein Irritation
4. Cardiac Arrhythmia
5. Fever
6. Diarrhea
7. Acute Leukemia
8. Skin Necrosis from Drug Infiltration

FIGURE 2.—Toxic effects of STZ.

dose schedule may have facilitated central nervous system uptake of STZ metabolites, resulting in these symptoms.

One instance of interaction between phenytoin and STZ has been noted (103). Use of these two drugs concurrently seemed to protect islet tumor cells from STZ cytotoxicity, thus preventing the therapeutic effect of STZ. These two drugs should not be used together.

A patient who received 67 g of STZ therapy over 28 months developed acute myelogenous leukemia (104). Second malignancies in the form of acute nonlymphocytic leukemia are well known to occur from prolonged therapy with a variety of alkylating agents, and it appears that STZ must be included in this list of drugs capable of inducing such a problem.

SUMMARY AND CONCLUSIONS

Figure 3 lists the tumors that are responsive or possibly responsive to STZ. STZ is the most effective single agent for the treatment of pancreatic islet cell cancers. It provides both tumor regression and biochemical improvement effects, with the latter being more frequent. All forms of functional or nonfunctional islet cell tumors are responsive, whether they are of beta cell or non-beta cell origin and regardless of the hormone being secreted. The combination of STZ and 5-FU appears more efficacious than STZ alone and usually has tolerable toxicity.

Carcinoid tumors are also responsive to STZ, but less frequently than islet cell cancers. Again, both objective tumor regression and biochemical improvement can be effected. Tumors of small bowel origin are more often responsive than tumors arising in other gastrointestinal sites or in the lung. The combination of STZ and 5-FU is active against these cancers, but whether it is more active than STZ alone is an unanswered question.

Other APUD cancers seem not to be affected by STZ. However, only in small cell carcinoma of the lung has STZ been adequately tested. Rare endocrine cancers have been studied for STZ efficacy,

CANCERS DEFINITELY RESPONSIVE TO STREPTOZOCIN

1. Pancreatic Islet (Beta or Non-beta) Cell Carcinomas
   a) Functional--Producing:
      Insulin
      Proinsulin
      ACTH
      Gastrin
      Glucagon
      Parathormone-like substance
      Alpha subunit HCG
      Somatostatin
      Vasoactive intestinal polypeptide
      Diarrheogenic hormones
      Calcitonin
      Serotonin
   b) Nonfunctional
2. Carcinoid Tumors

CANCERS POSSIBLY RESPONSIVE TO STREPTOZOCIN

1. Hodgkin's Disease
2. Colon Carcinoma
3. Pancreatic Ductal Adenocarcinoma
4. Hepatoma

FIGURE 3.—Tumors responsive to STZ.

but in too few cases to be certain of the lack of benefit.

STZ has been part of several multidrug regimens as "second-line" treatment of Hodgkin's disease. These regimens are reasonably effective, but there is no certainty that STZ is an essential component.

Multidrug regimens that include STZ have been reported by a few institutions to be more efficacious in both pancreatic and colon carcinomas than other known treatment for advanced disease. More work needs to be done, however, before any conclusions can be drawn regarding the role of STZ in these tumors.

STZ has been adequately studied in most other major cancer types, but in all cases there was negligible effect.

The major dose-limiting toxicity is renal. Nephrotoxicity can occur with one dose, but it is more commonly seen with repeated doses; it occurs in most patients if drug treatment is continued long enough. It is manifested by urinary sediment alterations and/or azotemia, with proteinuria being the most common finding. There is no known way of preventing nephrotoxicity, so all patients should have renal function monitored closely. If mild abnormalities appear, they will usually disappear with drug discontinuation.

Vomiting is the most unpleasant STZ side effect. Much greater patient acceptance could be achieved if effective means were found to minimize this problem.

Myelosuppression and hepatotoxicity are the only other potentially troublesome side effects. However, problems with either are uncommon.

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Rationale for the Use of Alternating Non-Cross-Resistant Chemotherapy[1]

J. H. Goldie,[2,3,*] A.J. Coldman,[2] and G. A. Gudauskas[2,4]

SUMMARY

In this paper an argument is presented for examining the rationale for the use of alternating non-cross-resistant chemotherapy. It is assumed that the development of a doubly resistant tumor cell (ie, a phenotype that is resistant to both arms of an alternating regimen) will result in incurability of the tumor. Thus, strategies that will minimize this risk can be expected to be the most effective. By means of a mathematical development and a computer program that simulates the process of doubly resistant cells appearing during treatment, it can be demonstrated that alternating the treatment regimen at every cycle will be the most effective strategy for a wide variety of cases.

[Cancer Treat Rep 66:439–449, 1982]

The emergence of drug-resistant tumor cells during cancer chemotherapy constitutes a formidable obstacle to achieving long-term remission or cure. There is considerable evidence that the selection for and overgrowth by drug-resistant cells is a major reason for treatment failure during experimental cancer chemotherapy (1–4). A limited number of observations in man together with inferences drawn from the behavior of clinical tumors that regress and recur during therapy suggest that many instances of clinical resistance are also associated with selection for drug-resistant variants (5–8). To minimize this problem, a number of investigators have employed alternating sequenced chemotherapy protocols (9–14). That is, where the number of effective agents for a tumor is greater than that which is feasible to give simultaneously, active combinations are alternated in some planned sequence. Variable results have been reported using this strategy, ranging from significant increases in response to essentially no change from standard therapy. In this paper we present an argument based on theoretic considerations which indicates that provided certain conditions are met, alternating chemotherapy at every cycle would be expected to be a more effective strategy to adopt.

THEORY

We have previously published a report of a model for the emergence of drug-resistant cells in tumors, which was based on the mechanism of somatic cell mutation (15). The essential features of this model are that mutations to resistance arise spontaneously and with a measurable frequency (the mutation rate). Because of the probabilistic nature of the process, there will be no unique value for the proportion of resistant cells in a family of tumors of a given size. That is, depending on whether the first mutations occur relatively early or late in the growth of the tumor, there will be a range of possible values for the size of the resistant fraction. There will also be a finite probability of there being no resistant cells present, and the value of this probability will be related to the mutation rate and inversely related to the size of the tumor. We have assumed that the

[1]Received Aug 14, 1980; revised Aug 81, 1981; accepted Sept 18, 1981.

[2]Division of Advanced Therapeutics (*J. H. Goldie* and *G. A. Gudauskas*) and Division of Epidemiology (*A. J. Coldman*), Cancer Control Agency of British Columbia, Vancouver, Canada.

[3]Department of Medicine, University of British Columbia, Canada.

[4]We thank Ms. Gillian Birt for secretarial assistance.

**Reprint requests to:* Dr. J. H. Goldie, Cancer Control Agency of British Columbia, 2656 Heather St, Vancouver, British Columbia, Canada, V5Z 3J3.

state of having zero resistant cells constitutes the minimum necessary condition for chemotherapeutic cure to be achieved. The value of P (probability of zero resistant cells) is given by:

$$P = \exp\{-\alpha(N' - 1)\}$$

where α is the mutation rate per cell generation, and N' is the tumor size (number of cells).

We have extended this basic model to incorporate the concept of having two resistant subpopulations (R_1 and R_2) that have arisen spontaneously and independently from the sensitive population in the tumor. We further assume that we have two treatments T_1 and T_2 (these could be single agents or combinations that are incorporated into a single protocol) which cannot be used simultaneously (because of toxicity). Treatment T_1 is effective against the sensitive (N) population and against resistant subpopulation R_2, and treatment T_2 is likewise effective against N and R_1. We are thus assuming there is no cross resistance between T_1 and T_2 and therefore incurability will occur when a doubly resistant phenotype occurs, which we will label as R_{1-2}.

A formula for calculating the probability of occurrence of the doubly resistant phenotype R_{1-2} is given in the Appendix. From this we see that the doubly resistant cells will appear more rapidly than would be expected from considering the simple product of the individual mutation rates. This process is illustrated schematically in figure 1. If a cell line has a constant probability α per cell generation of mutating to resistance (the mutation rate), then the average number of resistant cells in a tumor of N sensitive cells (assuming the resistant cells are kinetically identical to other cells) is given by (15):

$$R = \alpha N \ln(N)$$

If $\alpha = 10^{-5}$, when $N = 1 \times 10^4$, $R \cong 1$. Therefore, in a tumor of this size there will be an average of one resistant cell present.

Similarly, when there are R_1 resistant cells present there will be an expected

$$R_{1-2} = \alpha_2 R_1 \ln R_1$$

doubly resistant cells present that have arisen from cells which are resistant to T_1. Thus, where α =

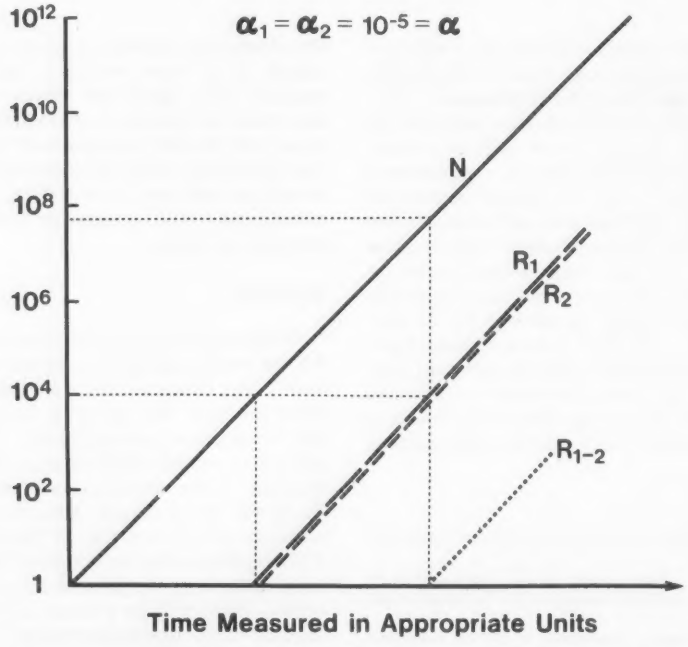

FIGURE 1.—R_1 and R_2 represent the plots of the mean No. of cells singly resistant to treatments 1 and 2. This illustrates that for this example, R_1 (= R_2) $\cong$ 1 when the sensitive tumor population (N) is 10^4. Therefore, when $R_1 = 10^4$, $R_{1-2} > 1$ and this occurs when $N \cong 10^8$.

10^{-5} and $R_1 = 1 \times 10^4$, $R_{1-2} \cong 1$ (ignoring mutations from R_2 to R_{1-2}). Now from above, $R_1 = \alpha N \ln N$ and thus by the acceleration of this effect we see that when $N = 10^8$, $R_{1-2} \cong 1$. Even though the product of the two mutation rates is 10^{-10}, we would expect an average of one doubly resistant cell present in a tumor of 10^8 cells.

We have developed a computer program that calculates the probability of doubly resistant cells appearing during the growth or regression of a tumor cell population. The basic assumptions underlying this model are similar to those that were used in developing the single resistant cell model that was described in an earlier publication (15). That is, that all the cells constituting the tumor have stem cell capacity, that each of the two resistant phenotypes (R_1 and R_2) arise with a constant probability of α per cell generation and that their kinetic behavior is identical to that of the parent sensitive cells, and that the emergence of a doubly resistant phenotype (R_{1-2}) constitutes a condition of incurability. We have also assumed that the doubly resistant phenotype emerges as a consequence of a two-step process (ie, $S \rightarrow R_1 \rightarrow R_{1-2}$), an assumption for which there is experimental evidence (4).

Treatment effects are simulated by algorithms that calculate the size of the residual tumor after it has been affected by one or the other of the two non-cross-resistant modalities that are available. In the case where the log-kill effect is assumed, for instance, the vulnerable cells in the population are reduced by a constant fractional amount at each application of treatment. The physical-chemical basis for the log-kill effect has been discussed by Skipper (16) and can be considered to be a measure of the probability of any one cell in the target population being killed following drug exposure. Increasing the dose of drug increases the probability of cell death, which translates into a greater log-kill effect. "Gompertzian" kill assumes that the amount of cell kill achieved is proportional to the instantaneous growth rate, and hence will vary throughout the biologic history of the tumor (17).

The computer program allows the examination of three models of tumor growth and regression upon administration of chemotherapy: (*a*) exponential tumor growth and log-kill treatment effects, (*b*) Gompertzian tumor growth and log-kill treatment effects, and (*c*) Gompertzian tumor growth and "Gompertzian"-kill treatment effects.

Treatment simulations utilizing the program will yield results in either a tabular or graphic form. In this paper we will, for convenience, utilize the first model (exponential growth and log-kill effect) for illustration.

Using the expressions developed in the Appendix, it is then possible to calculate the probability that a doubly resistant mutant will develop during the therapeutic program. Since the absence of such a mutant clone is assumed to be the minimal condition required for the tumor to be curable, this allows a quantification of the likely success of the treatment strategy. That is, the probability that no such clone arises is the probability that the tumor will ultimately be cured if the strategy is successful at eliminating the remaining tumor cells.

Execution of the program requires the specification of parameters that will establish the dynamics of the tumor system and the properties of the chemotherapeutic agents. These are specified as follows:

(*a*) The initial size of the tumor at first therapy.

(*b*) The doubling time for the tumor for the exponential model. The Gompertzian model assumes an instantaneous growth rate which is equal to that of the exponential model at the time of first therapy. The Gompertzian growth model further assumes that total malignant tumor cell burden cannot exceed 10^{13} cells.

(*c*) The interval between courses of therapy for each of the two therapeutic regimens.

(*d*) The log kill of each of the two therapies. For the Gompertzian regression model the initial therapeutic kill is assumed to be identical to that of the log-kill law at time of first therapy.

(*e*) The mutation rates to resistance for each of the two drug regimens.

The kill effect of each therapy is assumed to occur instantaneously upon its administration, and throughout the remaining period of the treatment interval, the tumor is assumed to regrow according to its specified kinetics. At the beginning of each treatment interval, either of the two therapies may be chosen. It is therefore an objective to sequence the available treatments in a way such that the tumor continuously regresses with no prolonged periods of regrowth and the probability is maximized that no doubly resistant cells will develop prior to eradication of the tumor.

To illustrate the process, consider the following idealized example. We will postulate a tumor of size $N = 10^{10}$ cells that has a doubling time of 36 days at the time treatment begins. In this case we will consider two treatments that are equally efficacious, ie, they have identical log kills of 2 and identical mutation rates of 10^{-6} and are given over the same interval of 21 days. The agents are assumed to be non-cross-resistant, ie, cells resistant to one agent have the same mutation rate to resistance to the other agent as do the purely sensitive cells and

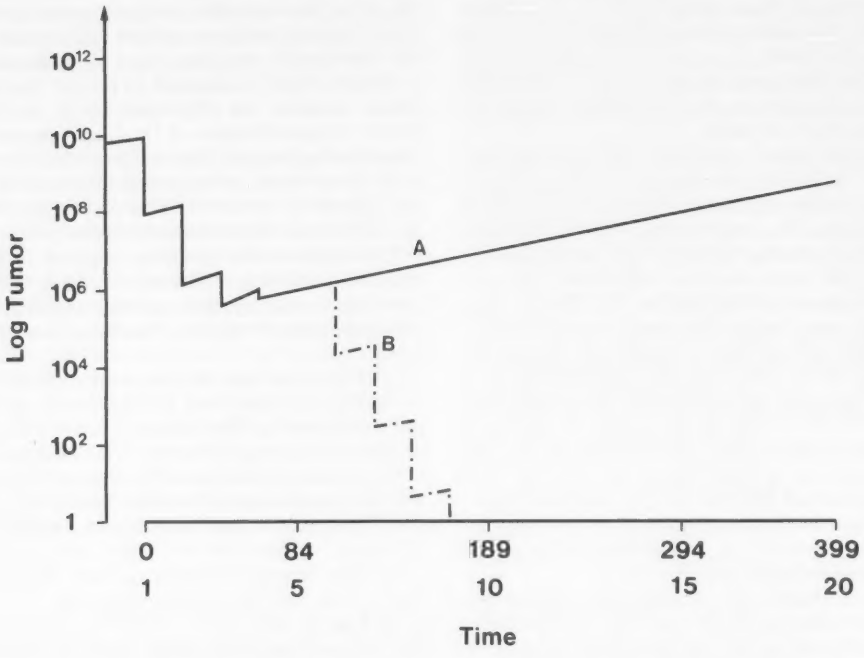

FIGURE 2.—Vertical axis represents S log tumor size and horizontal axis represents time in days (0–399) and No. of cycles of therapy (1–20). Curve A shows tumor size for the strategy of using treatment 1 (or equivalently treatment 2) continuously for parameters specified in text. First course of therapy occurs at Time 0. This strategy fails and tumor recurs. Curve B shows tumor size for the strategy where therapy is switched to treatment 2 for 4 courses, after 5 courses of treatment 1. After 9 courses, all sensitive cells and singly resistant cells have been eliminated. Probability that no doubly resistant cells have emerged prior to this point is 0.14. Thus, probability of total tumor elimination is 0.14.

the therapeutic kill of agent 1 against cells resistant to agent 2 is the same as for the purely sensitive cells and vice versa.

In this situation, exclusive use of either of the two agents is unlikely to result in cure since the probability is approximately 100% that tumor cells resistant to each of the two agents exist. However, cells that are resistant to both agents are much less likely to exist. Using the formula developed in the Appendix, the probability that there are no doubly resistant cells is given by

$$P = \exp\{-2\alpha_1\alpha_2(1 + N(\ln N - 1))\} = 0.64$$

Since we have assumed that absence of double resistance satisfied the minimal condition for curability, then there is a 64% chance that any tumor of size 10^{10} of the same type will be potentially curable. However, this probability is not fixed and will start to diminish after the start of therapy. As will be illustrated below, effective strategies will tend to stabilize the probability of cure at relatively high values, and ineffective ones will result in progressive diminution in this probability. To determine how this can be influenced, several different treatment strategies will be examined.

The first treatment protocol consists of 20 consecutive courses of treatment 1. As would be expected, this represents a failing strategy since a considerable number of tumor cells resistant to this treatment are already present. This is illustrated in figure 2 (in which the curve is plotted for the exponential model) where it is seen that a clinical remission (ie, $<10^8$ cells) has been quickly attained but after four cycles of therapy tumor regression ceases. At this point, the tumor is 100% resistant and proceeds to grow steadily in the face of further therapy to the point were it clinically recurs.

Clearly, once total resistance to one therapy is present, further use of this treatment is unproduc-

TABLE 1.—Probability of cure with treatment changed after 5 cycles*

Course No.	Treatment type	Proportion of cells resistant to—		Tumor size (No. of cells)	Probability of cure
		T_1	T_2		
—	—	2.30259×10^{-5}	2.30259×10^{-5}	1×10^{10}	0.6437
1	T_1	2.29775×10^{-3}	2.33769×10^{-5}	1.50172×10^{8}	0.5733
2	T_1	0.187193	1.94×10^{-5}	2.76108×10^{6}	0.4827
3	T_1	0.958386	1.00×10^{-6}	808,267	0.3731
4	T_1	0.999566	0	1.16114×10^{6}	0.2536
5	T_1	0.999996	0	1.73900×10^{6}	0.1422
6	T_2	1.0	0	26,056	0.1410
7	T_2	1.0	0	390	0.1410
8	T_2	1.0	0	6	0.1410
9	T_2	0	0	0	0.1410

*Values relate to the time immediately prior to beginning of next course of therapy.

tive, so it would be better to utilize the second therapy thereafter. This strategy is illustrated in figure 2, where after five courses of agent 1 and four courses of agent 2, the tumor appears to be eradicated. This will only have occurred if no doubly resistant cells have emerged. Table 1 gives the breakdown of these probabilities for this strategy; the overall probability that no doubly resistant cell has emerged either prior to or during therapy is 14%. Thus, there is a 14% probability that the tumor will have been eradicated and the therapy successful.

Table 1 also shows that during the period of continuous treatment with agent 1 the probability of no double resistance declines rapidly and stabilizes after the start of administration of agent 2. Also, after the third course of agent 1 the tumor moves into a growth phase for the last two cycles of therapy 1. This suggests that changing the treatments at this point would result in a greater therapeutic effect than persisting with the five courses of treatment 1.

Table 2 presents information for a strategy in which therapy is switched after three courses of each agent. We see that this strategy has the desired effect as the probability of zero doubly resistant cells is stabilized at a higher level. After three courses of each therapy there are very few cells remaining and a further course of each therapy is sufficient to eradicate the remaining tumor. Figure 3 illustrates this approach. However, when the therapy is alternated after every three cycles it is clearly suboptimal since the last two courses of agent 1 have no effect. But this tactic is superior to the previous strategies since there is now a 37% probability that the tumor will have been eradicated.

Obviously there is room for improvement since the best we can do with any strategy (given the constraints of our problem) is to achieve a 64% probability of cure. Table 2 shows a situation similar to that in table 1, namely that the probability of cure falls rapidly during continued therapy with agent 1 and stabilizes after agent 2 is instituted. This suggests that switching therapies earlier may prove to be beneficial.

A strategy of changing treatments after each cycle of therapy is illustrated in table 3 and figure 3. This results in only a small diminution in the probability of zero doubly resistant cells from the theoretic

TABLE 2.—Probability of cure with 3-course alternating regimen

Course No.	Treatment type	Proportion of cells resistant to—		Tumor size (No. of cells)	Probability of cure
		T_1	T_2		
—	—	2.30259×10^{-5}	2.30259×10^{-5}	1×10^{10}	0.6437
1	T_1	2.29775×10^{-3}	2.33769×10^{-5}	1.50172×10^{8}	0.5733
2	T_1	0.187193	1.94×10^{-5}	2.76188×10^{6}	0.4827
3	T_1	0.958386	1.00×10^{-6}	808,267	0.3731
4	T_2	0.95829	1.00×10^{-4}	12,112	0.3716
5	T_2	0.949	1×10^{-2}	183	0.3716
6	T_2	0.50	0.50	6	0.3716
7	T_1	1.0	0	4	0.3716
8	T_1	1.0	0	6	0.3716
9	T_1	1.0	0	9	0.3716
10	T_2	0	0	0	0.3716

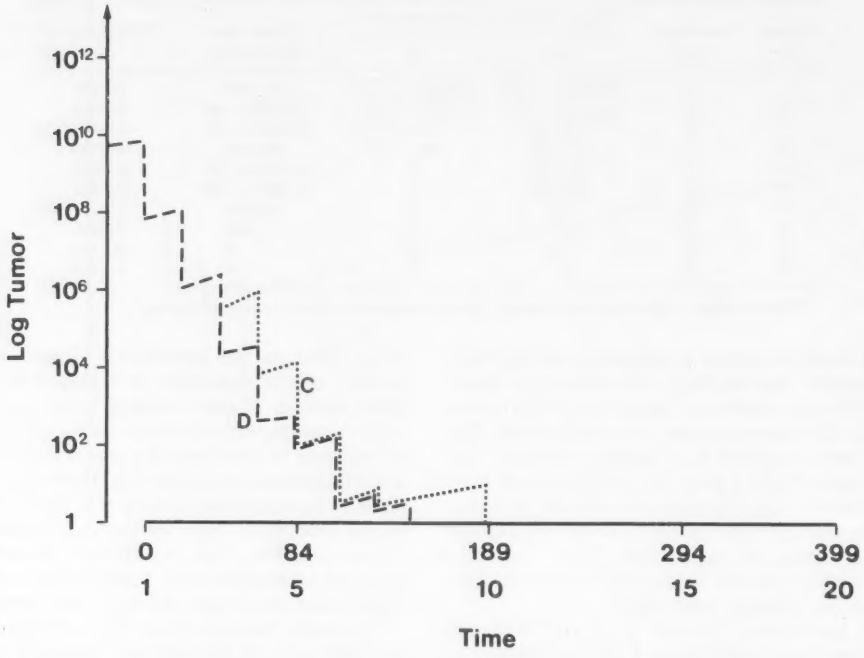

FIGURE 3.—Curve C shows tumor size for the strategy where 3 cycles each of treatment 1 and treatment 2 are repeated alternately. This is seen to result in elimination of sensitive and singly resistant tumor cells after 10 cycles of therapy. Probability of total tumor elimination is 0.37. Curve D represents tumor size for the strategy where therapy is alternated at each cycle. This results in elimination of the sensitive and singly resistant cells after 8 cycles of therapy. Probability of total tumor elimination is 0.57.

maximum of 64%. Furthermore this loss occurs almost totally during the first cycle of therapy, and is independent of which agent is used first. As table 3 indicates, at the time the last treatment is applied the tumor consists entirely of cells resistant to T_1. This last alternation then extinguishes the second remaining compartment of resistant cells and eradicates the tumor. It should be emphasized that this is the expected outcome in approximately three fifths of cases. In the other two fifths of cases, at least one doubly resistant cell would be present (either at the start of or developing during treatment) and would result in treatment failure. The growth of these doubly resistant cells is not depicted in graphic

TABLE 3.—Probability of cure with 1-course alternating regimen

Course No.	Treatment type	Proportion of cells resistant to—		Tumor size (No. of cells)	Probability of cure
		T_1	T_2		
—	—	2.30259×10^{-5}	2.30259×10^{-5}	1×10^{10}	0.6437
1	T_1	2.29775×10^{-3}	2.33769×10^{-5}	1.50171×10^{8}	0.5733
2	T_2	2.29×10^{-3}	2.33×10^{-3}	2.25525×10^{6}	0.5713
3	T_1	0.18687	1.90×10^{-3}	41,461	0.5698
4	T_2	0.1573	0.1600	738	0.5697
5	T_1	0.949	9.7×10^{-3}	183	0.5697
6	T_2	0.50	0.50	6	0.5697
7	T_1	1.0	0	4	0.5697
8	T_2	0	0	0	0.5697

form, but the actual frequency distribution of such cells would be highly variable (15). Thus, there would be no unique pattern to treatment failure.

DISCUSSION

All results presented here are for an exponentially growing tumor in which therapy produces log kills (ie, the number of cells killed is a linear dose-dependent fraction of the number of cells present). Although Gompertzian kinetics for tumor growth are often assumed, and many animal solid tumors exhibit this form, it is still nonetheless far from clear whether clinical tumors exhibit Gompertzian kinetics (18,19). In addition, we believe that the exponential model has a number of advantages as a tool for simulation studies of tumor treatment. Thus, the total tumor burden present may represent the number of tumor cells located at many sites. For the exponential model, the growth and behavior of the tumor dynamic are unaffected by compartmentalization into a number of various-sized cellular aggregates. However, for other models of tumor growth that exhibit retardation with increasing size, it would be necessary to make a number of ad hoc assumptions about the size and frequency distribution of the various component tumor colonies. In this model we are assuming that systemic effects resulting in general growth retardation do not occur. In addition, we feel that the weight of experimental evidence, both in animal tumors (and in in vitro human systems) favors the log-kill effect as being the basis for tumor reduction by cytotoxic agents (20–23). This is best demonstrated by cell cycle stage-nonspecific agents that have a log-linear dose-response effect. Dose-response curves for cell cycle stage-specific agents are more complex, with an initial steep dose-response effect followed by a plateau. To a first approximation, however, a fractional cell kill in the range of one to four logs can be assigned to such agents (21).

Further simulations using a variety of growth and other treatment parameters reveal an essentially uniform picture of the superiority of sequential alternating non-cross-resistant therapy over other strategies, assuming that mutations to resistance do occur within the tumor. That this should be true for a broad variety of cases is not surprising if one considers the behavior of each of the individual resistant subpopulations. To employ any treatment strategy that permits one of the resistant subtypes to grow for any significant period of time unimpeded by therapy is to greatly increase the likelihood of further mutations arising within that subtype. Strategies that will act to reduce each resistant subtype in tandem will minimize the risk of further mutations.

We have, however, identified situations in which alternation at every cycle might not prove to be the optimal strategy. Such exceptions arise because the values we have used in these calculations are averages and the behavior of real systems will obviously be greatly variable. Indeed, this basic variability is predicted using this approach (15). Thus, there would be a nonnegligible possibility in a given tumor that, by chance, one resistant fraction would be considerably larger than the other, even if the values for their mutation rates were identical. To commence by alternating the treatment courses would allow the larger of the two resistant subpopulations to have intervals when it would grow unimpeded. Since it is already large in absolute numbers, further increases in size of this population would greatly increase the probability of mutation to double resistance. If the tumor burden could be monitored, then this circumstance would quickly manifest itself as minimal or absent therapeutic response to one of the treatment arms. Should this occur, then this particular treatment would need to be modified or abandoned. In the treatment of subclinical disease, such assessment of tumor burden might not be feasible, and here one would need to rely on the statistical likelihood that such cases would be much less apt to occur than those where the sizes of the resistant populations were not significantly different.

In this admittedly theoretic and idealized model of tumor therapy, a number of critical assumptions have been made. In the example given, certain values for the mutation rates were assumed. Although this clearly has implications for the quantitative superiority of one strategy compared to another, changing these values will not affect the qualitative superiority of alternation. Furthermore, we have assumed that the resistant cells will exhibit the same kinetic behavior as the other tumor cells. Relaxation of this assumption to permit the two resistant compartments to behave differently will lead to situations where alternation is no longer superior. A complete lack of cross resistance has been postulated. Examination of the appropriate relationships presented in the Appendix illustrates that relaxation of these assumptions does not necessarily lead to differing conclusions. The essential quantity that must be maintained is the symmetry between the two treatments, ie, that they have similar mutation rates to single resistance, similar mutation rates to double resistance, the same log kill on the sensitive cells, and the same log kills to the single resistant compartment; in addition, the singly resistant subpopulations must have the same growth curves. When

there are substantial deviations from this symmetry, then alternating between the treatment arms may not be the optimal therapeutic approach. Likewise, we are assuming it is not possible to give the two treatments simultaneously. Clearly, in the absence of antagonistic effects or excessive toxicity, as many agents as possible should be given concurrently. Indeed, the superiority of simultaneous administration of multiple agents follows directly from the previous considerations.

In clinical practice two generally equivalent and non-cross-resistant modalities are not often readily available. Alternating-sequence therapies that do not incorporate such modalities may well fail to show therapeutic advantage. Hodgkin's disease is one case in which two nearly equivalent and non-cross-resistant treatments appear to be available. Alternating ABVD with MOPP in this disease has been reported to produce definitely superior results to either modality used alone (14). A number of other clinical trials, although still in preliminary form, also show an apparent advantage in alternating between treatment arms (12,13,24,25). While other explanations for this effect may be possible, we feel that there is significance to the fact that alternating chemotherapy so readily emerges as a superior strategy when the phenomenon of mutation to resistance is assumed to be a major limiting factor in cancer chemotherapy.

APPENDIX

Let N be the size of the sensitive population, and we will assume that the tumor grows continuously from unicellular origins.

Let α_1 and α_2 be the mutation rate to resistance of treatments 1 and 2, respectively.

Let R_1 and R_2 be the expected size of the population of cells resistant to treatments 1 and 2, respectively.

When there are N_0 sensitive cells, there are $R_1(N_0)$ cells resistant to treatment 1; then at some later time when there are N sensitive cells, there will be

$$R_1(N) = R_1(N_0)\frac{N}{N_0} + \alpha_1 N \ln(N/N_0) \qquad \text{(i)}$$

cells resistant to treatment 1 (15).

If the mutation rate to resistance is the same for mutations from single to double resistance as it is for sensitive to single resistance (ie, α_1, α_2), then we may calculate the probability of having zero doubly resistant cells. We will further assume that cells cannot mutate to double resistance without initially becoming resistant to treatment 1 or 2 first.

First consider mutations from cells resistant to treatment 1 to double resistance. Let $P^0_{1-2}(N_2/N_1)$ be the probability that no doubly resistant mutants will be present in a tumor of size N_2 given that there were none present at size N_1. Mutations to R_{1-2} (doubly resistant population) from R_1 will result from the growth of R_1. However, in any period some of the growth in R_1 will be as the result of new mutations from N. These new mutations cannot contribute to growth of R_{1-2}, and thus the growth in R_1 (as N grows from N to N + ΔN, for ΔN small), which can contribute to R_{1-2}, is given by

$$R_1(N + \Delta N) - R_1(N) - \alpha_1 \Delta N \cong \left(\frac{dR_1}{dN} - \alpha_1\right)\Delta N$$

From (15) the probability that there will be no doubly resistant mutants during this small increase in growth of R_1 is

$$P^0_{1-2}((N + \Delta N)/N) = \left(1 - \alpha_2\left(\frac{dR_1}{dN} - \alpha_1\right)\Delta N\right)$$

Thus,

$$P^0_{1-2}((N + \Delta N)/N) = P^0_{1-2}(N/N)\left(1 - \alpha_2\left(\frac{dR_1}{dN} - \alpha_1\right)\Delta N\right)$$

Now, approximating $P^{o}_{1-2}((N + \Delta N)/_{N})$ by $1 + \frac{dP_{1-2}}{dN}\Delta N$

and solving the resulting differential equation, we have

$$P^{o}_{1-2}(N_2/N_1) = \exp\{-\alpha_2[R_1(N_2) - R_1(N_1) - \alpha_1(N_2 - N_1)]\}$$

Likewise, we can construct a similar expression for mutations from the second resistant subpopulation.

Thus, the probability that there are no doubly resistant cells present at size N_2, given that there were none at size N_1, is

$$\exp[-\alpha_2[R_1(N_2) - R_1(N_1)] - \alpha_1[R_2(N_2) - R_2(N_1)] + 2\alpha_1\alpha_2(N_2 - N_1)] \qquad \text{(ii)}$$

For the special case $N_1 = 1$, $R_1(1) = R_2(1) = 0$

$$\exp\{-2\alpha_1\alpha_2[1 + [\ln N - 1]N]\}$$

The above equations (i and ii) allow the calculation of the probability of zero doubly resistant cells under a variety of growth and regression circumstances, assuming mean values for the computed entities. The computer program uses these relationships, employing the various kinetics dictated by the respective growth and regression models. Application of therapy is assumed to have the effect of instantaneously reducing the size of the tumor by the amount required, and throughout the remaining treatment interval the tumor will regrow. As an example we will illustrate the detailed calculations involved in producing the quantities shown in table 1 where the tumor has been treated with one course of T_1. *See* text for the tumor and treatment parameters.

Prior to T_1 number resistant to T_1 $= 2.30259 \times 10^5$
Prior to T_1 number resistant to T_2 $= 2.30259 \times 10^5$
Prior to T_1 number sensitive $= 1 \times 10^{10}$

After T_1 number resistant to T_1 $= 2.30259 \times 10^5$
After T_1 number resistant to T_2 $= 2.303 \times 10^3$
After T_1 number sensitive $= 1 \times 10^8$

After 21 days of regrowth in an exponentially growing tumor with a doubling time of 36 days, the number of sensitive cells $= 1.4983 \times 10^8$.

Using equation (i), the number of cells resistant to T_1 is given by

$$R_1(N) = R_1(N_0)\frac{N}{N_0} + \alpha_1 N \ln(N/N_0)$$

$$= 2.30259 \times 10^5 \times 1.4983 + 10^{-6} \times 1.4983 \times 10^8 \ln(1.4983)$$
$$= 344{,}998.67 + 60.5819$$
$$= 3.45059 \times 10^5$$

Similarly,

$$R_2(N) = 2.30259 \times 10^3 \times 1.4983 + 10^{-6} \times 1.4983 \times 10^8 \ln(1.4983)$$
$$= 3449.99 + 60.5819$$
$$= 3510.57$$

Thus, the total tumor size is

$$= 1.4983 \times 10^8 + 3.45059 \times 10^5 + 3.5106 \times 10^3$$
$$= 1.5018 \times 10^8$$

Proportion resistant to T_1

$$= \frac{3.45059 \times 10^5}{1.5018 \times 10^8} = 2.297 \times 10^{-3}$$

Proportion resistant to T_2

$$= \frac{3510.57}{1.5018 \times 10^8} = 2.338 \times 10^{-5}$$

Now, using equation (ii) we have

$$\text{prob} = \exp\{-10^{-6}[R_1(N_2) + R_2(N_2) - R_1(N_1) - R_1(N_2)] + 2 \times 10^{-12}[N_2 - N_1]\}$$
$$= \exp\{-10^{-6}[3510.6 - 2302.6 + 345{,}057 - 230{,}259] + 2 \times 10^{-12}[1.4983 \times 10^8 + 1 \times 10^{-4}]\} = 0.8905$$

Thus, the probability of cure = $0.6437 \times 0.8905 = 0.5733$.
Further calculations are performed in a similar fashion. Differences between these calculations and those in the table merely reflect errors in rounding off.

For each interval of regrowth we can calculate the probability of no doubly resistant cells occurring, and thus multiplying these probabilities together gives the required probability for the strategy.

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Multiple Myeloma Resistant to Melphalan: Treatment With Doxorubicin, Cyclophosphamide, Carmustine (BCNU), and Prednisone[1]

Robert A. Kyle, Thomas F. Pajak, Edward S. Henderson, Ismat U. Nawabi, Kurt Brunner, Patrick H. Henry, O. Ross McIntyre, and James F. Holland[2,*]

SUMMARY

Sixty-five patients with multiple myeloma resistant to melphalan were randomized to receive cyclophosphamide, doxorubicin (Adriamycin), and prednisone (CAP) (30 patients) or carmustine (BCNU), doxorubicin, and prednisone (BAP) (35 patients). Objective responses occurred in two patients in the CAP group and in seven in the BAP group. Indirect responses were noted in seven additional patients in the CAP group and in six additional patients in the BAP group. Toxic effects consisted mainly of leukopenia and thrombocytopenia. Median survival did not differ between the two treatment groups (CAP, 8.4 months; BAP, 7.7). Objective responders had a longer survival than nonresponders (14.5 vs 7.7 months).

[Cancer Treat Rep 66:451–456, 1982]

Although melphalan is well-established in the treatment of multiple myeloma (1–6), resistance to melphalan often develops in patients who have had initially satisfactory responses, as well as in some patients who have not responded. Cyclophosphamide also has given beneficial results in the treatment of myeloma (7–9), and patients with myeloma resistant to melphalan have improved when treated with cyclophosphamide (10–12). Carmustine (BCNU), a nitrosourea, which may act as an alkylating agent, has been effective in the treatment of hamsters with plasmacytomas (13). Significant benefit also has been obtained with the nitrosoureas in a number of advanced mouse tumors refractory to treatment with most antineoplastic agents (14). Responses to BCNU have been demonstrated in the treatment of multiple myeloma (12,15,16).

In one study, doxorubicin (Adriamycin) produced responses in three of 23 patients with multiple myeloma (17). In another study, doxorubicin produced objective benefit in three of nine patients with myeloma resistant to melphalan and prednisone (18). However, only one of 13 patients with far-advanced multiple myeloma resistant to standard agents obtained any benefit with doxorubicin (19). A combination of doxorubicin and BCNU produced improvement in seven of 13 patients with myeloma who were unresponsive or who had relapsed after chemotherapy (20). Six of 14 patients with multiple myeloma resistant to melphalan and prednisone obtained objective responses when treated with BCNU, cyclophosphamide, doxorubicin, and prednisone (21).

Prednisone added to melphalan produced a better response than did melphalan alone in good-risk patients with multiple myeloma (3). Poor-risk patients with multiple myeloma also responded significantly when prednisone was added (22).

[1]Received Aug 18, 1980; revised Sept 1, 1981; accepted Sept 18, 1981.

[2]This study was performed by the Cancer and Leukemia Group B (*James F. Holland*, chairman) and was supported in part by Public Health Service grants CA-04646, CA-16118, CA-02599, CA-12046, CA-04326, and CA-04457 from the National Institutes of Health, Department of Health and Human Services. The following members participated in the study: *R. A. Kyle* (Mayo Clinic, Rochester, MN); *T. F. Pajak* (Cancer and Leukemia Group B, Statistical Office, Scarsdale, NY); *E. S. Henderson* (Roswell Park Memorial Institute, Buffalo, NY); *I. U. Nawabi* (State University of New York, Downstate Affiliated Hospitals, Brooklyn); *K. Brunner* (Swiss Group, Bern, Switzerland); *P. H. Henry* (University of Missouri, Columbia); *O. R. McIntyre* (Dartmouth Medical School, Hanover, NH); and *J. F. Holland* (Mount Sinai School of Medicine, New York, NY).

**Reprint requests to:* Dr. Robert A. Kyle, Hematology and Internal Medicine, Mayo Clinic, Rochester, MN 55901.

The major objective of this study was to determine whether patients resistant to melphalan would respond to a combination of agents that have been of some benefit when used separately and in combination in multiple myeloma: specifically, whether such patients would respond better to a combination of cyclophosphamide, doxorubicin, and prednisone (CAP) or to a combination of BCNU, doxorubicin, and prednisone (BAP).

MATERIALS AND METHODS

Criteria

All patients fulfilled the criteria for the diagnosis of multiple myeloma (23). Resistance to melphalan was defined as lack of objective improvement after at least 3 months of adequate therapy or as progression of myeloma after an initial response to melphalan (12).

Study criteria required the completion of any irradiation at least 3 weeks before acceptance in the study. Previous low-dose corticosteroid treatment did not disqualify the patient, but previous exposure to any of the nitrosoureas, cyclophosphamide, or doxorubicin was unacceptable. Patients with congestive heart failure were excluded. Patients presenting with azotemia, hypercalcemia, or acute infections received maximal supportive therapy prior to entry. Leukopenia (< 2500 cells/mm^3), thrombocytopenia ($< 75{,}000$ cells/mm^3), or marrow hypoplasia was a contraindication unless these abnormalities resulted from progression of the myeloma. Randomization was not done until after recovery from previous melphalan-induced cytopenias. The laboratory determinations initially and during the study were the same as previously reported (11).

Evaluation of Response

Objective response was defined as (*a*) a $\geq 50\%$ reduction from the initial serum M protein concentration; (*b*) a $\geq 50\%$ reduction in urinary M protein excretion from an initial value of ≥ 1.0 g/24 hours; (*c*) a $\geq 50\%$ decrease in the maximal area of measured soft tissue masses, without the appearance of new lesions; or (*d*) recalcification of bone lesions without the development of new lesions during the study (24). A patient satisfying any of these four criteria was classified as an objective responder. Indirect response was defined as an increase in hemoglobin or hematocrit, an improvement of performance score, or a decrease of plasma cells (12).

Treatment Regimens

Patients who fulfilled the criteria for admission to the study were randomly assigned to either of two treatment regimens. In the CAP regimen, doxorubicin (30 mg/m^2) and cyclophosphamide (400 mg/m^2) were given iv every 3 weeks. In the BAP regimen, doxorubicin (30 mg/m^2) and BCNU (75 mg/m^2) were given iv on Day 1. On Day 22, patients received doxorubicin (30 mg/m^2) alone. The cycle was repeated every 6 weeks. Thus, doxorubicin was given every 3 weeks and BCNU was given every 6 weeks. In both regimens, prednisone (0.6 mg/kg) was given in three equally divided doses daily for 7 days every 3 weeks.

Leukocyte and platelet counts were determined every 3 weeks. The doses of doxorubicin, cyclophosphamide, and BCNU were altered according to the leukocyte and platelet counts (11). When cytopenias occurred and the bone marrow was hypercellular, full doses of medications were given, but when the bone marrow was hypocellular, the doses of these drugs were reduced. If no leukopenia or thrombocytopenia had occurred in a previous course, the doses were increased by increments of 25%. The use of cyclophosphamide was discontinued if cystitis occurred, and the use of doxorubicin was stopped in the event of congestive heart failure or persistent arrhythmias. Doxorubicin was discontinued at a cumulative dose of 500 mg/m^2.

RESULTS

Of the 72 patients entered in the study, seven were disqualified because of incomplete records (two), unavailability of records (one), improper randomization (two), and ineligibility (two). Of the remaining 65 patients (table 1), 30 received CAP and 35 received BAP. Three patients who received CAP and two who received BAP were included only for evaluation of toxicity and for survival analysis because M protein was absent from the serum and urine.

Examination of individual parameters at entry to the study revealed no important differences in the percent of males and females, hemoglobin level, percent of bone marrow plasma cells, serum creatinine level, leukocyte count, or median time from diagnosis of multiple myeloma to entry in the CAP and BAP groups. Compared with patients in the BAP group, patients in the CAP group were slightly older, more of them had performance scores of 0, and more had slightly higher median platelet counts; however, these differences were not significant (table 2). The pretreatment levels of serum calcium in patients assigned to the CAP regimen were signifi-

TABLE 1.—Sources of evaluable patients

Institution	No. of evaluable patients
MAYO*	9
RPMI*	7
SUNY*	6
SWISS*	6
MO*	5
MCV*	4
NYH	3
LIJMC	3
UCSD	3
DMS	2
TJUH	2
MCGILL	2
MSH	2
PRETORIA	2
BOSTON	2
TUFTS	2
VERMONT	2
RIH	1
WRAMC	1
BCRC	1

*Affiliations of authors of this study.

cantly lower than the levels in patients assigned to the BAP regimen. However, the frequency of patients in the CAP group with an initial serum calcium level > 12.0 mg/dl was not significantly different from the frequency of such patients in the BAP group (3% vs 17%; $P = 0.28$).

Seven patients (22%) in the BAP group and two (7%) in the CAP group had objective responses ($P = 0.22$). If the treatment comparison is restricted to patients with initial serum calcium levels < 12.0 mg/dl, the result remains virtually unchanged (BAP, 23%; CAP, 7%). The observed difference in the frequencies of objective responses between the two treatment programs lies chiefly in a statistical trend seen in the patients who had responded previously to melphalan (table 3). Five (28%) of 18 such patients achieved objective responses to BAP, whereas none of 14 similar patients responded to CAP ($P = 0.08$). Two of nine patients who had failed to respond previously to melphalan and prednisone achieved objective responses to CAP, while two of ten similar patients responded to BAP.

The nine patients who had objective responses are no longer in the study. Six patients had progression of their myeloma after an initial objective response and were removed from the study after 174, 184, 317, 343, 384, and 675 days. Three patients died while in the study from sepsis (despite a normal leukocyte count), myocardial infarction, and acute nonlymphocytic leukemia.

Nine patients (28%) in the BAP group and eight (29%) in the CAP group had indirect responses. Three of the patients in the BAP group and one in the CAP group also had objective responses. The frequency of individual parameter responses is shown in table 4. In summary, 32% of the patients receiving CAP and 41% of the patients receiving BAP achieved some response.

TABLE 2.—Pretreatment characteristics of CAP and BAP groups

Characteristic	Therapy	
	CAP	BAP
No. of patients	30	35
% males	57	58
Age (yrs)		
Median	63	58
% patients > 70	27	16
Serum calcium (mg/dl)		
Median	9.3	10.2
% of patients with > 12.0	3.0	17.0
Median		
Hemoglobin (g/dl)	9.6	10.0
Leukocyte count (cells/mm³)	3700	3800
Platelet count (cells/mm³)	152,000	122,000
Bone marrow (% plasma cells)	50	52
Urine M protein (g/24 hrs)	1.2	2.0
Serum M protein (g/dl)	4.3	4.0
No. of patients with M protein		
IgG	17	15
IgA	8	12
Light chain only	2	6
No M protein	3	2
% of patients with—		
Bone lesions	77	85
Performance score		
0	20	9
1–2	60	64
3–4	20	27

Median survival did not differ between the two treatment groups (BAP, 8.4; CAP, 7.7 months; $P = 0.75$; fig 1). As might be expected, the objective responders had a longer survival than the nonresponders (14.5 vs 7.7 months; $P = 0.04$; fig 2). This difference was seen only during the first 20 months of treatment.

The percent of chemotherapy given in both regimens amounted to approximately 75% of the full-schedule dose. Leukopenia or thrombocytopenia accounted for the reductions in dose.

Toxic effects consisted mainly of leukopenia and thrombocytopenia. The median leukocyte count nadirs were 2300/mm³ (Day 40) with CAP and 2200/mm³ (Day 33) with BAP. The median platelet count nadirs were 86,000/mm³ (Day 35) with CAP and 80,000/mm³ (Day 36) with BAP. Five patients in the CAP group and four in the BAP group had leukocyte count nadirs > 3000/mm³ and platelet count nadirs > 100,000/mm³.

TABLE 3.—Objective response frequencies by prior melphalan response

Prior melphalan response	No. of patients evaluated	Objective response	
		No. of patients	% of patients
	CAP		
No response	9	2	22
Response	14	0	0
Unknown	5	0	0
Total	28	2	7
	BAP		
No response	10	2	20
Response	18	5	28
Unknown	4	0	0
Total	32	7	22

Severe infections, mainly pneumonia or septicemia, occurred in seven patients who received CAP and in five patients who received BAP. Only two of these 12 patients had a leukocyte count > 2000/mm³ at the onset of the infection. Drug toxicity was the probable cause of leukopenia, which produced fatal infections in two patients.

One patient had myocardiopathy, but he had received < 100 mg/m² of doxorubicin; there was no proof that the drug was responsible. A 70-year-old woman was removed from the study because of congestive heart failure and an abnormal echocardiogram. She had received 275 mg/m² of doxorubicin.

DISCUSSION

The treatment of patients with multiple myeloma resistant to melphalan is difficult and frustrating. Although melphalan is effective in approximately two thirds of patients, nearly all eventually become resistant to the drug and relapse.

In patients initially resistant to melphalan, CAP and BAP produced objective response frequencies of 7% (two of 28) and 22% (seven of 32), respectively. The difference in these response frequencies was not statistically significant ($P = 0.22$). However, BAP showed a higher objective response frequency in patients who had previously responded to mel-

TABLE 4.—Parameter response

Parameter	No. of patients evaluated	Response	
		No. of patients	% of patients
	CAP		
M protein			
Serum	28	1	4
Urine	12	2	17
Soft tissue	2	0	0
Bone	22	0	0
Hemoglobin	23	0	0
Performance	15	5	33
Bone marrow	26	4	15
	BAP		
M protein			
Serum	24	2	8
Urine	17	5	29
Soft tissue	1	0	0
Bone	27	0	0
Hemoglobin	31	2	6
Performance	17	2	12
Bone marrrow	30	7	23

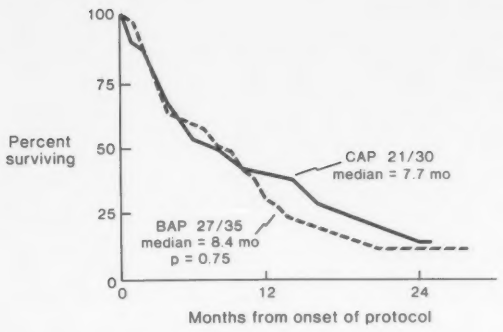

FIGURE 1.—Survival of patients with multiple myeloma treated with CAP or BAP.

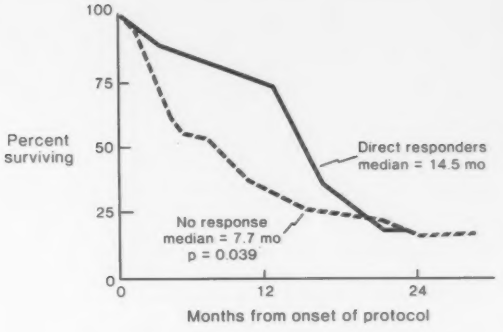

FIGURE 2.—Survival of patients with multiple myeloma according to response to therapy.

phalan than in similar patients treated with CAP (28% vs 0; $P = 0.08$). The frequency and severity of hematologic toxic effects as well as the amount of drug delivered were similar for the two combinations. Finally, there was no difference between them in duration of survival.

The doses of doxorubicin, BCNU, and cyclophosphamide in this study may have been conservative, but 12 (18%) patients developed severe infections; two were fatal. The compromised bone marrow of patients with resistant myeloma who have received previous alkylating agents and possibly radiation therapy precluded larger doses of chemotherapy without unduly increasing the frequency of severe infections or bleeding.

It is difficult to compare our results with some more optimistic reports in the literature (20). The definition of resistance to melphalan, as well as differences in pretreatment patient characteristics and in drug scheduling, may largely account for these discrepancies.

In a previous study of 89 evaluable patients with multiple myeloma resistant to melphalan, three (6.4%) of 47 patients receiving cyclophosphamide plus prednisone obtained an objective response, while seven (16.7%) of 42 responded to the combination of cyclophosphamide, BCNU, and prednisone (12). These results are not significantly different from those of the current study, in which doxorubicin was added. The two protocols had the same patient eligibility criteria, except that patients with cardiac disease were excluded from the current study. Pairwise comparison of sex, age, hemoglobin, number of plasma cells in the bone marrow, size and type of M protein in the serum and urine, presence of bone lesions, performance score, and initial leukocyte and platelet counts, as well as the time from diagnosis to entry to the protocol, showed remarkable similarity of pretreatment characteristics. The only discrepancy was a median serum calcium level of 10.2 mg/dl in the BAP group, compared with 9.3 mg/dl ($P = 0.01$) in the BCNU, cyclophosphamide, and prednisone group of the previous protocol (12).

In two studies of the Cancer and Leukemia Group B involving 89 and 65 evaluable patients with multiple myeloma refractory to melphalan, BCNU given as a component of combination chemotherapy appeared to add increments of 10% and 15% to the objective response rates, compared with current cyclophosphamide-containing combinations that served as controls. When these two studies are combined, the BCNU-containing combinations have significantly higher objective response rates than combinations without BCNU (19% vs 7%; $P = 0.04$). These findings support the inclusion of BCNU in combinations for melphalan-refractory multiple myeloma, with an expectation of higher activity than that resulting from the use of cyclophosphamide alone.

Two historical comparisons can be made to evaluate the effects of doxorubicin in melphalan-resistant patients. The addition of doxorubicin to cyclophosphamide and prednisone or the substitution of doxorubicin for cyclophosphamide with BCNU and prednisone added little or nothing to the frequency of objective response (17% for BCNU, cyclophosphamide, and prednisone vs 22% for BAP; 7% for cyclophosphamide and prednisone vs 7% for CAP).

Ideally, the evaluation of BCNU or doxorubicin should be done in a single randomized trial in which sample sizes are large enough to detect a meaningful clinical difference with sufficient statistical power. However, in melphalan-resistant myeloma, in which the objective response frequencies for at least one regimen may be rather low (5%–15%), it is almost impossible to execute such trials, because of ethical considerations and/or rapidly declining investigator interest in continuing the trial. However, when one

utilizes historic comparisons, as we did, they must be viewed with caution because many factors besides the differences in protocol treatment may explain the differences in clinical outcome.

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Hemolytic-Uremic Syndrome Associated With Mitomycin Therapy[1,2]

Michael D. Pavy,[3] Elizabeth L. Wiley,[4,5] and Martin D. Abeloff[3,*]

SUMMARY

Two patients who developed the hemolytic-uremic syndrome after mitomycin therapy are reported. Pathologic examination showed renal changes consistent with hemolytic-uremic syndrome and nuclear changes in kidney secondary to chemotherapy. The evidence for mitomycin nephrotoxicity is discussed. The two patients reported may represent a variation of drug toxicity different from previously reported minor creatinine increases associated with mitomycin administration.

[Cancer Treat Rep 66:457–461, 1982]

The hemolytic-uremic syndrome (HUS) is a clinical condition which results from a variety of pathophysiologic mechanisms. Diagnostic features include renal failure characterized by renal microangiopathy involving small arterioles and glomerular capillaries, microangiopathic hemolytic anemia (MAHA), and a consumptive thrombocytopenia (1). HUS is seen most commonly in children following a diarrheal prodrome; it varies in its severity and has a mortality rate < 5% (2). The HUS has been reported to occur in association with pregnancy (3), oral contraceptives (4), and renal transplantation (1); in addition, there appears to be a familial association (5). These forms of HUS are more common in adults and are associated with a mortality rate of approximately 30%. Recurrent episodes of HUS have also been reported (6). We report here two patients who developed HUS after administration of mitomycin for advanced malignancy.

[1]Received Feb 6, 1981; revised Aug 26, 1981; accepted Sept 18, 1981.

[2]Supported in part by Public Health Service grant CA-06973 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]The Johns Hopkins Oncology Center, The Johns Hopkins University School of Medicine, Baltimore, MD.

[4]Department of Pathology, The Johns Hopkins University School of Medicine, Baltimore, MD.

[5]*Present address:* Department of Pathology, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL.

**Reprint requests to:* Martin D. Abeloff, MD, The Johns Hopkins Oncology Center, 600 N Wolfe St, Baltimore, MD 21205.

CASE REPORTS

Patient 1

A 21-year-old white male presented with a 3-week history of a mass in the right supraclavicular fossa. Biopsy of the mass revealed an anaplastic carcinoma without mucin production. Evaluations for primary lesions included thyroid scan, iv pyelogram, thoracic and abdominal computerized axial tomographic (CAT) scans, bronchoscopy, and extensive ear, nose, and throat examination; all results were negative. Biomarkers including α-fetoprotein, CEA, and β-human chorionic gonadotropin were negative. The patient was treated with 5000 rads to the bilateral cervical regions and supraclavicular fossae, with complete resolution of the mass. Three months after radiotherapy a superior vena caval syndrome developed and chest X-ray showed a new right paratracheal mass. At that time, cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) chemotherapy was begun. This resulted in complete resolution of the superior vena caval syndrome and paratracheal mass. CHOP was continued for five cycles and then therapy was changed to 10 mg/m^2 of mitomycin and 6 mg/m^2 of vinblastine given every 3 weeks. After a 3-month period the patient had received a total of 92 mg of mitomycin and appeared to be in complete remission. Chemotherapy was discontinued and laboratory studies showed a hematocrit of 40%, a wbc count of 4700/mm^3, a platelet count of 118,000/mm^3, a BUN of 15 mg/dl, and a creatinine of 1.2 mg/dl.

Two months after the last dose of mitomycin, he was noted to have a hematocrit of 25%, a corrected reticulocyte count of 5%, a wbc count of 4100/mm^3, a platelet count of 53,000/mm^3, a BUN of 20 mg/dl, and a creatinine of 1.9 mg/dl. Urinalysis showed 4+ proteinuria, 10 rbcs/high power field, and many granular casts. Peripheral blood smear showed fragmented rbcs and decreased platelets. Bone marrow examination revealed a cellular marrow, with erythroid hyperplasia and increased megakaryocytes. Evaluations for vasculitis, disseminated intravascular coagulation, recurrent cancer, and infectious etiology were all negative. Rbc and platelet transfusions resulted in no significant increment in hematocrit or platelet count, and the clinical diagnosis of adult HUS was made. Hemodialysis was begun; however, the patient continued to have a severe MAHA with thrombocytopenia, and died of hyperkalemia 4 months after renal dysfunction was first noted.

Autopsy findings demonstrated no evidence of carcinoma. The pleural and pericardial surfaces had fibrinous exudates and adhesions. The mediastinum contained fibrous scarring. The bone marrow was obscured by autolytic changes but was cellular with all marrow elements present. Sections of liver and lung showed atypical parenchymal cells with intranuclear cytoplasmic projections. The kidneys were small (80 and 90 g) and smooth externally. Microscopic examination demonstrated many glomeruli containing hyaline thrombi plugging capillaries and afferent arterioles. Other glomerular capillaries were thickened with swollen endothelial cells (fig 1). Mesangium was also increased in many glomeruli. Mesangial cells were frequently swollen and bizarre, having large vesicular nuclei and occasional intranuclear cytoplasmic projections (fig 2). Tubular atrophy was present. Virtually every artery showed endothelial proliferation. Most vessels had marked reduction in lumen caliber (fig 3). Immunofluorescence staining showed only weak immunoreactivity to fibrin in glomerular capillary loops and absence of immunoglobulin deposition.

Patient 2

A 59-year-old white male presented with a 6-month history of back pain. Chest X-ray disclosed a right upper lobe lung mass. Bronchoscopy revealed adenocarcinoma consistent with a lung primary. A CAT scan of the abdomen revealed bilateral enlarged adrenal glands consistent with adrenal metastases. Hematocrit was 41.2%, wbc count was 8500/mm^3, platelet count was 323,000/mm^3, creatinine was 1.2 mg/dl, and BUN was 13 mg/dl. He started to receive 20 mg of mitomycin and 12 mg of vinblastine every 3 weeks. Because of significant nausea and vomiting, the chemotherapy was stopped after five cycles

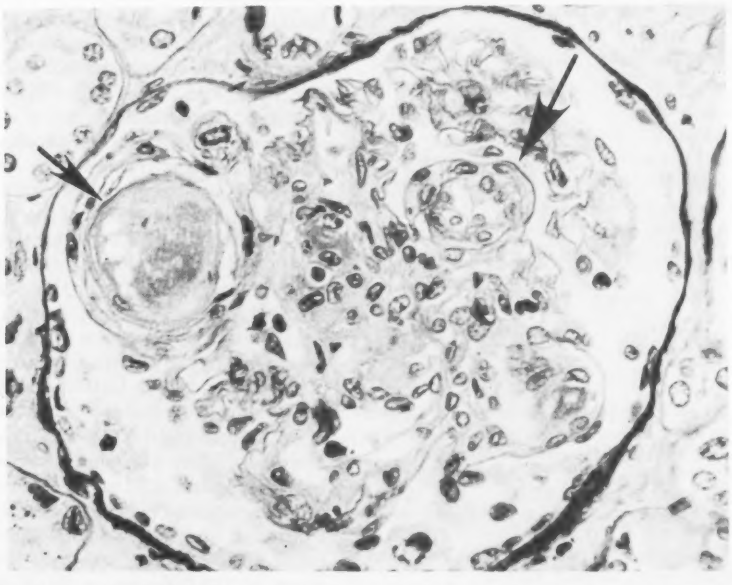

FIGURE 1.—Section of kidney from autopsy of patient 1 showing a glomerular capillary plugged with hyaline thrombus (small arrow) and thickened capillary loops (large arrow) (PAS-MS, × 350).

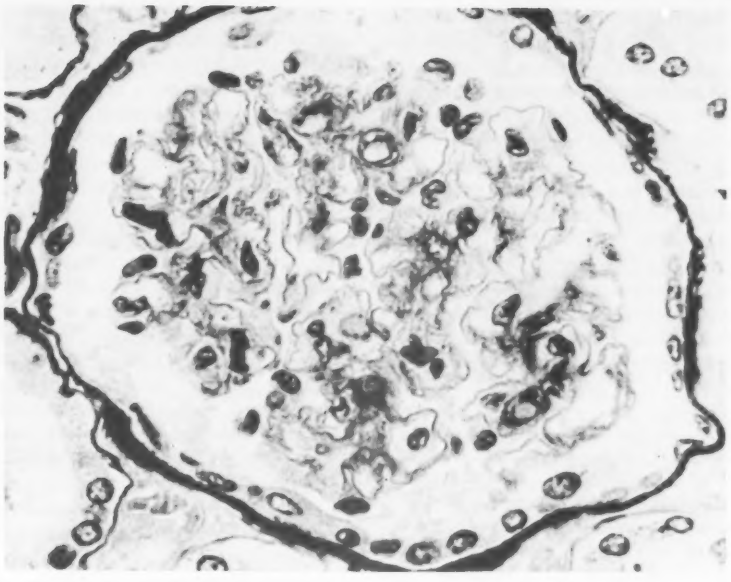

FIGURE 2.—Glomerulus from kidney in fig 1 with swollen mesangial cells having large vesicular nuclei and intranuclear cytoplasmic projections (PAS-MS, × 350).

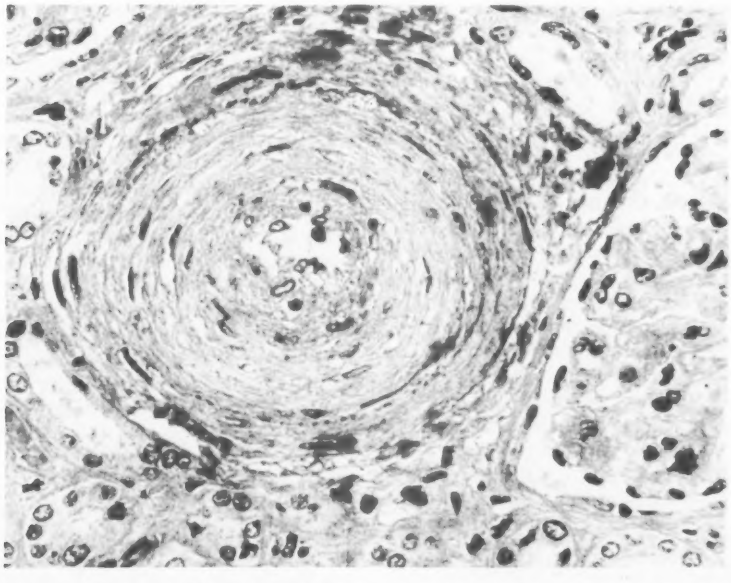

FIGURE 3.—Small artery from kidney in fig 1 shows marked endothelial proliferation and decreased lumen caliber (PAS-MS, × 200).

and the patient was followed in a partial remission. Twelve weeks after discontinuation of chemotherapy the patient was noted to have a hematocrit of 21%, a corrected reticulocyte count of 8%, a wbc count of 6400/mm^3, and a platelet count of 64,000/mm^3. The blood smear showed microangiopathic rbc changes, and the bone marrow was cellular with erythroid hyperplasia and increased megakaryocytes. The creatinine had increased to 3.6 mg/dl and the BUN had increased to 65 mg/dl. Retrograde pyelogram, prothrombin time, serial fibrinogens, and fibrin split products were normal. The creatinine continued to increase, the MAHA persisted, severe hypertension developed, and the patient died at home of an intracerebral hemorrhage. Permission for autopsy was refused.

DISCUSSION

MAHA is not uncommon in cancer patients. It may be seen with disseminated intravascular coagulation, sepsis, or overwhelming carcinomatosis. However, the development of HUS in association with carcinomatosis is not well-recognized, although renal abnormalities may be seen with tumor emboli to the kidneys (7). In both of our patients, extensive evaluations for coagulopathy and infection were negative. The autopsy findings of patient 1 showed no residual malignancy, and the clinical picture of patient 2 was not that of disseminated carcinomatosis. The HUS which these two patients developed may likely have been a manifestation of mitomycin toxicity. Although both patients also received vinblastine, there are no reports of nephrotoxicity from this agent, to date.

Mitomycin is an antitumor antibiotic which has been associated with nephrotoxicity. Animal studies have shown the drug to cause papillomatous proliferation of ureters and tubular necrosis (8,9). These types of renal pathology have not been seen in man, and the renal changes of the HUS have not been noted in mitomycin-treated animals.

The early reports of mitomycin renal toxicity by Liu et al (10) described glomerular changes, nuclear pleomorphism, and intranuclear inclusions similar to those present in the first patient presented. The nuclear pleomorphism has been described in the HUS; the intranuclear inclusions which were present not only in the kidneys but also in the liver and lungs are most probably cytotoxic drug effects since the changes have been demonstrated in patients receiving drugs that directly inhibit DNA synthesis. In the report of Liu et al, there is no mention of MAHA although one patient was found to have a chromium-51 rbc half-life of 5½ days, associated with thrombocytopenia and renal insufficiency. Krauss et al (11) described one patient with MAHA, disseminated intravascular coagulation, and renal failure which may have been due to disseminated carcinomatosis or mitomycin treatment. Recently, Gulati et al (12) reported two patients who developed MAHA, thrombocytopenia, and renal failure associated with mitomycin administration. Renal pathologic changes were compatible with HUS. It appears that the above reports and our two case reports may represent an unusual form of mitomycin nephrotoxicity.

In addition to these cases, there is some evidence to support mitomycin as a mild nephrotoxin in man. The Southwest Oncology Group and the group at Wayne State University have reported clinical nephrotoxicity with mitomycin (13). During the past 3 years, we have treated 94 patients having a variety of solid tumors with mitomycin at The Johns Hopkins Oncology Center. The total cumulative doses of mitomycin ranged between 20 and 100 mg. Four patients developed unexplained renal insufficiency (ie, creatinine $\geq$ 2.0 mg/dl). Two of these patients were reported here as examples of HUS. The other two patients had transient mild creatinine elevation not associated with significant clinical problems.

Despite these retrospective analyses, the clinical significance of mitomycin nephrotoxicity has not been adequately quantitated. The two patients reported here are unusual because of the severe renal insufficiency and associated MAHA. It is interesting to hypothesize a possible idiosyncrasy or genetic predisposition to the development of the HUS in some patients given mitomycin. A recent report of thrombotic thrombocytopenic purpura and HUS developing in HLA-identical siblings suggests a possible genetic predisposition to these diseases (14).

Mitomycin is presently being used in advanced breast carcinoma (15), gastric carcinoma (16), and recently has been advocated for treatment of "adenocarcinoma of unknown primary" (17). The possibility of a drug-induced severe HUS leading to irreversible renal disease should be considered as more patients are treated with this agent. The period of observation for renal dysfunction should include not only the time during drug administration but also a significant period following discontinuation of treatment.

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Tumor Size, Response Rate, and Prognosis in Small Cell Carcinoma of the Bronchus Treated by Combination Chemotherapy[1,2]

P. G. Harper,[3] R. L. Souhami,[3,*] S. G. Spiro,[4] D. M. Geddes,[5] M. Guimaraes,[5] F. Fearon,[3] and J. F. Smyth[3,6,7]

SUMMARY

Ninety-eight patients with small cell carcinoma of the bronchus (SCCB) were treated with a combination of cyclophosphamide, methotrexate with calcium leucovorin rescue, and lomustine (CCNU). VP-16-213 was given on relapse. Response rate and survival were analyzed according to extent of tumor, presence of the syndrome of inappropriate antidiuretic hormone production (SIADH), and, in 38 patients, size of intrathoracic tumor as measured by computerized tomographic (CT) scanning. The median survival of the entire group was 34.6 weeks. In patients with limited disease the median survival was 46 weeks compared to 29 weeks for those with extensive disease. This difference in survival between patients with limited and extensive disease was most marked in those showing a partial response (median survival, 45.9 and 28 weeks, respectively). Patients with ectopic antidiuretic hormone production had a poor prognosis even if they had achieved a complete response. Only one partial response to VP-16-213 occurred. There was a strong correlation between initial intrathoracic tumor size as assessed by total cross-sectional area on CT scan and response and survival. When the total area exceeded 30 cm^2 there were no complete responders, but there were complete responses in eight of 21 patients when the area was < 30 cm^2. The relationship between intrathoracic tumor size and response rate was present when analyzed according to disease extent, age, sex, and performance status. A clear relationship between total tumor area and survival was also found. When SCCB is treated with chemotherapy alone, the prognosis is better for those patients who present with limited disease, without SIADH, and with small intrathoracic tumor. In this group it is possible that further intensification of therapy may result in prolonged survival.

[Cancer Treat Rep 66:463–470, 1982]

Small cell carcinoma of the bronchus (SCCB) is a rapidly growing (1), early and widely metastasizing tumor (2); untreated, patients have a median survival of only a few months (3,4). Combination chemotherapy has increased the median survival of patients treated either with chemotherapy alone (5) or with additional radiotherapy to the primary site (5).

[1]Received Feb 12, 1981; revised Aug 31, 1981; accepted Sept 29, 1981.

[2]Supported by a grant from the Cancer Research Campaign.

[3]Department of Radiotherapy and Oncology, University College Hospital, London, England.

[4]University College and Brompton Hospitals, London, England.

[5]London Chest and Brompton Hospitals, London, England.

[6]*Present address*: Medical Oncology Unit, Western General Hospital, Edinburgh, Scotland.

[7]We thank the following physicians for referring cases: Drs. T. J. H. Clark, S. W. Clarke, K. M. Citron, P. Cole, M. Hodson, E. E. Keal, M. Green, J. V. Collins, W. P. U. Kennedy, P. J. D. Heaf, P. D. Davies, M. Erooga, J. R. Govan, D. Eraut, D. O. McCarthy, P. Storring, K. Greenacre, D. Leaver, D. Phillips, M. Gumpel, J. Milledge, A. J. Newman-Taylor, and Professor M. Turner-Warwick. Statistical analysis was performed by the Research Centre for the Mathematical Modelling of Clinical Trials at Warwick University.

**Reprint requests to*: Dr. R. L. Souhami, Department of Radiotherapy and Oncology, University College Hospital, Gower St, London WC1E 6JJ, England.

Using conventional staging methods, patients in whom disease is confined to one hemithorax (limited disease) appear to have a higher response rate to chemotherapy and prolonged survival compared to those with more extensive disease (6–8). We have shown that these staging methods underestimate the extent of disease in both thorax and abdomen and are inaccurate methods of assessing the extent of the primary tumor (9). If tumor size is related to response rate and prognosis, the number of patients with a small tumor in a study will influence the observed results.

Disease-free survival > 2 years is now reported in 5%–13% of patients (10–12). Hansen et al (13), using a combination of cyclophosphamide, methotrexate, and lomustine (CCNU), obtained a response rate of 56%. Cohen et al (14) compared the response rates when the same drugs were used in either a high dose without modification for hematologic toxicity or a lower dose. The higher dose increased the response rate from 56% to 96%, and in 30% of the patients there was a complete disappearance of all tumor. The number of patients studied was, however, small, and some of the improved survival could be accounted for by the larger proportion of patients with limited disease in the high-dose group.

The present study was devised with the following aims: (*a*) to make a more accurate assessment of the relationship between tumor size and prognosis using computerized tomographic (CT) scanning; (*b*) to assess the response to the three drugs used by Cohen et al (14) but with the dose of methotrexate increased to 200 mg/m² as a 24-hour infusion followed by calcium leucovorin rescue; and (*c*) to gain additional information on VP-16-213, administered when patients relapsed, since single-agent studies had previously shown this drug to have a response rate in SCCB of up to 50% (15) when used as initial treatment.

MATERIALS AND METHODS

Between November 1977 and April 1979, 98 previously untreated patients with SCCB were admitted to the study. Patients were excluded only if they were > 75 years old, if they had other cancers in addition to SCCB, or if their serum creatinine was > 124 μmols/liter (1.8 mg/100 ml). Once they were accepted in the study all patients were included in the analysis. The median age of the patients was 62 years (range, 31–75); there were 68 males and 30 females. The histologic diagnosis was based on material obtained at bronchoscopy for 66% of the patients; mediastinoscopy, 5%; thoracotomy, 4%; lymph node biopsy, 9%; percutaneous needle biopsy of the lung alone, 9%; and positive sputum cytology on three occasions, 7%. Using the Eastern Cooperative Oncology Group (ECOG) scale, the pretreatment performance score (PS) was 0–1 for 40.8% of the patients; 2, 44.9%; 3, 10.2%; and 4, 4.1%. In addition to full physical examination, all patients had the following staging procedures: chest radiograph (posteroanterior and lateral); cbc; plasma urea; electrolytes; creatinine; calcium; phosphate; alkaline phosphatase; total protein; albumin; SGOT; γ-glutamyl transpeptidase; cortisol; bone marrow aspirate and trephine biopsy; bronchoscopy; and isotope scan of bone, brain, and liver. This group of "conventional" staging procedures was supplemented in 50 consecutive patients by CT scans of the thorax and abdomen, using an EMI 5005/2085 whole-body scanner. Slices (13-mm thick) were taken at 2-cm intervals from thoracic inlet to pelvic brim, with a scanning time of 20 seconds for each slice. Approximately 20–24 slices were taken. The contribution of CT scans to staging has been reported previously (9). To conform with reported studies in SCCB we have described disease confined to one hemithorax, including mediastinal lymph nodes and ipsilateral supraclavicular lymph nodes, as "limited" disease. Any disease beyond this is termed "extensive" disease.

Assessment of Intrathoracic Tumor Size

In 38 of the 50 patients in whom CT scans were obtained, tumor margins could be clearly delineated from adjacent lobar collapse or consolidation of the lung. Using the program available on the CT scanner for measurement of irregular areas, the tumor present on each slice was outlined and the area was determined in square centimeters. The sum of the tumor areas on each slice was taken as the total tumor area for each patient. All measurements were made on three separate occasions without knowledge of the patients' names or prognoses. In the remaining 12 patients (24%), no clear tumor margin could be delineated and therefore no estimate of tumor area could be obtained.

Chemotherapy

Combination chemotherapy was given every 21 days until relapse. Methotrexate at a dose of 200 mg/m² was given as a 24-hour infusion, with calcium leucovorin rescue started 2 hours following the infusion. Rescue consisted of calcium leucovorin at doses of 9 mg iv every 6 hours × 4 and 15 mg orally every 6 hours × 4. Cyclophosphamide was given as an iv bolus injection of 1 g/m² reduced to 750 mg/

m^2 from course 3. Lomustine was given orally at a dose of 100 mg/m^2 in courses 1 and 3 *only*. On relapse, patients were treated with 120 mg/m^2 of VP-16-213 iv as a 30-minute infusion on Days 1, 3, and 5 of each 21-day cycle for three courses or until further disease progression. On subsequent relapse, no further chemotherapy was given.

Dose Adjustment

Treatment was postponed if the total wbc count was < 3000/mm^3, if the neutrophil count was < 1000/mm^3, or if the platelet count was < 100,000/mm^3. Blood transfusions were given for hemoglobin < 9.5 g/dl but chemotherapy was not postponed for anemia alone.

Assessment of Results

All patients were restaged following the fourth course of chemotherapy. A complete response (CR) was defined as the disappearance of all tumor as judged by physical examination, chest X-ray, isotope scans, and blood and biochemical parameters. A partial response (PR) was defined as a reduction in the size of all measurable lesions by at least 50% of the greatest tumor diameter. Any response less than this or not evaluable was considered to be no response. Survival curves have been plotted and log-rank tests have been applied as recommended by Peto et al (16).

RESULTS

Survival Related to Staging Investigations

All of the 98 patients treated with chemotherapy were evaluable for response. The overall response rate was 74%. Seventeen patients (18%) achieved a CR and 55 (56%) achieved a PR. Median survival for the entire group was 34.6 weeks (fig 1). Forty patients (41%) had limited disease as judged by conventional staging procedures and 58 (59%) had extensive disease. The patients with limited disease lived longer than those with extensive disease (fig 1) (median survival, 46 vs 29 weeks); however, these differences did not reach statistical significance overall ($P = 0.15$). When this difference was analyzed in more detail it was found that if a CR was obtained, there was no significant difference in median survival between patients with limited (70.6 weeks) and extensive (59.4 weeks) disease ($P > 0.83$). This was also the case if no response was observed at all (limited disease, 27.5 weeks; extensive disease, 17.0 weeks; $P > 0.43$). However, in the partial responders there was a significant difference in median survival in favor of those patients with limited disease (45.9 vs 28 weeks for extensive disease; P

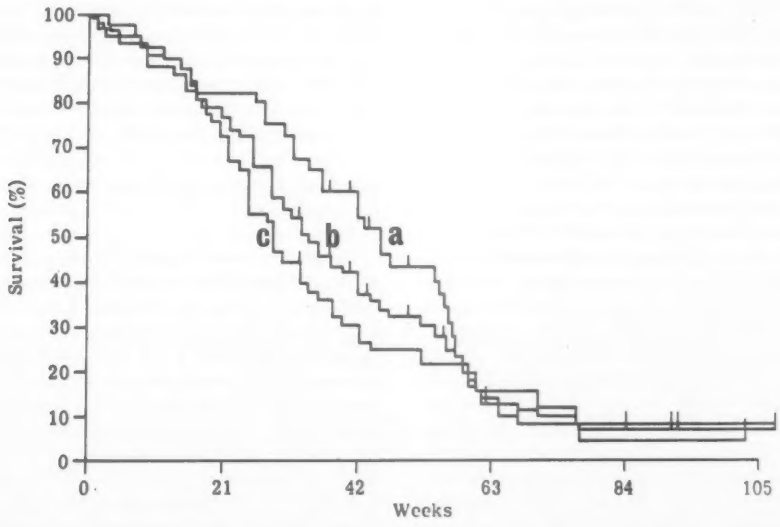

FIGURE 1.—Actuarial survival curve related to extent of disease at presentation. a = limited disease; b = all patients; c = extensive disease.

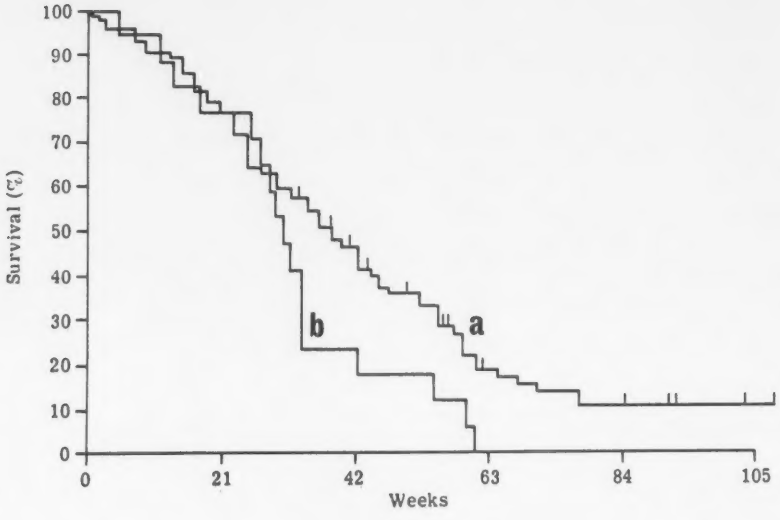

FIGURE 2.—Actuarial survival curve related to SIADH. a = without SIADH; b = with SIADH. $P = < 0.05$.

< 0.05). The modest difference in survival in favor of the limited disease category is therefore accounted for by the improvement in survival in the partial responders.

A consistently low plasma sodium level (< 130 mmols/liter) accompanied by an inappropriately high urine osmolality with the absence of hypotension, dehydration, or uremia was used as the definition of the syndrome of inappropriate antidiuretic hormone production (SIADH). This was present in 16 patients. Median survival of these 16 patients was significantly less than that of the 82 remaining patients (31 vs 38.3 weeks; $P < 0.05$) (fig 2). Further analysis of the survival of these patients with SIADH showed that the difference in survival lay in the limited-disease group. In the six limited-disease patients with SIADH the median survival was 30.0 weeks compared to 46 weeks in the 34 limited-disease patients without SIADH. In the extensive-disease patients the median survival was 31.6 weeks for both groups. No patients with SIADH lived > 61 weeks, compared to 22% overall. Four of the patients with SIADH achieved a CR with the disappearance of all electrolyte disturbance in addition to regression of tumor from all other sites. The median survival of these four patients when compared to all other patients achieving a CR was notably poor (31.7 vs 67.6 weeks; $P < 0.001$). The patients with this syndrome achieving a CR survived no longer than the patients who did not respond (31.7 vs 30.5 weeks).

Initial patient PS has repeatedly been shown to be a significant prognostic indicator. In the 98 patients survival was correlated with the ECOG score: median survivals for PS groups 1, 2, 3, and 4 were 47.5, 35.7, 30, and 8 weeks, respectively. Among the patients with SIADH there was a smaller number with a PS of 1, suggesting that there is an interrelationship between SIADH, PS, and survival, but the numbers are too small for further analysis.

Survival Related to Tumor Size Assessed by CT Scan

Fifty consecutive patients were assessed by CT scans of the thorax and abdomen in addition to conventional staging procedures. Using the TNM system (17), 27 (77%) of 35 patients conventionally staged as having localized T_1 or T_2 tumors had their stage increased to T_3 (extensive tumors) (9). The median survival of 58.8 weeks for the remaining eight patients with T_2 tumors was significantly longer than the median survival of 34 weeks for the 42 patients with T_3 tumors ($P < 0.001$). CT scans led to the detection of involved subcarinal nodes in an additional 30% of the patients, unsuspected adrenal masses in 16%, and retrocrural nodes in 8%. By conventional staging, 37 patients (74%) were found

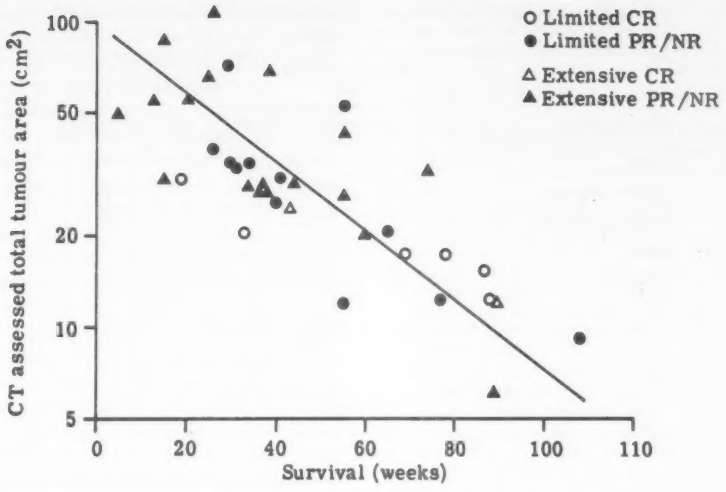

FIGURE 3.—Relationship between survival, response, and total cross-sectional area of intrathoracic tumor (cm^2).

to have stage III tumors; 47 patients (94%) were found to have stage III tumors following CT scan.

Further assessment of the size of the primary tumor and adjacent lymph nodes was possible in 38 of the 50 patients. Patients were included in this analysis irrespective of whether they had limited or extensive disease. The relationship between total tumor area and survival is shown in figure 3. A highly significant correlation was found ($P < 0.001$) between intrathoracic tumor size and survival, patients with small tumors surviving longer. Tumor size was also shown to be related to response rate. Taking an arbitrary cut-off point of 30 cm^2 in total area, a clear difference was shown in the response rate in small tumors compared to large tumors (table 1). All of the complete responders had smaller tumors and there were more limited-disease patients in this group. The trend towards worse response in patients with large tumors was also found when the group of 38 patients was analyzed with respect to stage, sex, PS, and age (table 2).

Response to VP-16-213 During Relapse

Twenty-six patients received at least three courses of VP-16-213 at a dose of 120 mg/m^2 iv on Days 1, 3, and 5 every 21 days. The treatment was well-tolerated but seven patients had one course postponed by 1 week because of a total wbc count < 3000/mm^3. Nine patients had never responded to the initial chemotherapy and the other 17 had relapsed following either a CR (four patients) or a PR (13 patients). There was only one partial responder to VP-16-213, and no complete responders. An additional 12 patients received less than three courses of VP-16-213. They showed no evidence of remission and died before the planned minimum of three courses could be completed.

TABLE 1.—Total intrathoracic tumor area assessed by CT scan in 38 patients, related to extent of disease and response

	Total tumor area*			
	> 30 cm^2		< 30 cm^2	
	CR	PR and no response	CR	PR and no response
Limited disease	0	6	6	5
Extensive disease	0	11	2	8
Total	0	17	8	13

*Values = No. of patients.

TABLE 2.—Response related to mean total intrathoracic tumor area assessed by CT scan

Patient group	Mean tumor size (cm^2)		Regression slope
	CR	PR and no response	
Total	23.9	39.6	−4.30
Limited disease	23.3	29.7	−3.67
Extensive disease	24.2	47.5	−4.39
Males	25.8	38.1	−3.41
Females	21.5	41.2	−5.23
ECOG PS			
0–1	23.8	34.5	−3.54
2–3	24.0	42.6	−4.35
Age (yrs)			
0–61	23.0	44.6	−3.96
62+	25.0	35.7	−4.38

Additional Treatment

Radiotherapy was given as palliative treatment to 43 patients for brain metastases, superior vena caval obstruction, persistent hemoptysis, or painful bone lesions following failure of chemotherapy.

Toxicity

Treatment was generally well-tolerated. Nausea and vomiting were controlled by antiemetic agents and were severe in only five patients. Due to hematologic toxicity, 44 courses of chemotherapy were delayed for ≥ 1 but < 2 weeks, and 19 courses were delayed for ≥ 2 weeks. Systemic infection occurred in five patients and responded to parenteral antibiotics in all. Other side effects encountered were hemorrhagic cystitis in one patient and severe mucositis in two.

DISCUSSION

The study provides useful information about staging and tumor size in relation to prognosis, response rates, and survival. The TNM staging system has not been found to be useful in prognosis of this disease because early dissemination is so common. The rough categorization into limited and extensive disease on the basis of the usual staging procedures has, however, been of some prognostic value. In this study we have shown, as have others (6–8,14,18), that limited disease has, overall, a rather better prognosis than extensive disease. However, this is accounted for almost entirely by improvement in survival in the partial responders. This is perhaps not surprising since the initial extent of the disease as judged by the usual staging procedures may only be a small factor in survival in patients with either very sensitive or totally insensitive tumors.

The increasing sophistication of staging procedures has resulted in patients with relatively small amounts of metastatic disease now being entered in the extensive-disease category. Such changes have caused the terms limited and extensive disease to be somewhat less useful as prognostic indicators than was previously thought.

In the search for an indicator of good prognosis we have carefully measured initial intrathoracic tumor size by the most sensitive method currently available, the CT scan. Tumor size has been calculated from multiple cross-sectional areas. Although obviously imprecise, the data have shown an unequivocal relationship between intrathoracic tumor size and survival. CR to chemotherapy was found only in tumors < 30 cm^2 in total area, and the larger tumors also tended to be extensive, having detectable metastases. Furthermore, the few patients with small intrathoracic tumors who had extrathoracic metastases usually had only one site of spread. A small intrathoracic tumor as judged by CT scans therefore put the patient into a good prognostic category. This relationship between response rate and tumor size holds true when the data are analyzed with respect to disease extent, age, sex, and PS. Survival in the eight patients who were shown to have T_2 tumors by CT scanning was clearly better than in the patients with T_3 tumors.

The study has also shown that presence of SIADH was associated with a poor prognosis, particularly in patients with limited disease. Somewhat surprisingly, even the four patients with SIADH who achieved a CR had a poor prognosis. This suggests, although the numbers are small, that the syndrome is associated with a tumor where drug resistance emerges quickly.

PS has consistently been shown to be a good prognostic indicator irrespective of stage, and, despite changes in staging method, continues to be an important variable in all randomized studies.

When the study was started the results obtained by Cohen et al (14) were among the best then published. Our response rates and median survivals do not equal those reported by Cohen et al (14) using the same agents. There seems to be no advantage to using the higher dose of methotrexate. In a randomized study, Arnold et al (19) have recently showed no benefit in response or survival in increasing the methotrexate dose from 30 mg/m² to 1 g/m², and Maurer et al (20) also reported no difference between 30 and 250 mg/m², their results being very similar to ours. A second difference between our study and the induction regimen of Cohen et al (14) is that we used lomustine in cycles 1 and 3 only. This seems unlikely to account for the difference since response rates to lomustine are low when it is used as a single agent (18). It is frequently observed that large studies in cancer chemotherapy tend to produce lower response rates than those obtained with smaller studies. Many factors may be responsible, including patient selection and criteria of response. It should be emphasized that no patient, once accepted for this study on the criteria indicated, was excluded from the analysis.

VP-16-213 has been shown to be an effective agent in the initial treatment of SCCB, with a mean response rate of 35% (15,21). Cohen et al (22) and Hansen et al (23) reported that patients who had become resistant to treatment with cyclophosphamide, methotrexate, and lomustine still responded to VP-16-213. Our results, which will be reported in detail elsewhere, do not confirm this and indicate that there is cross-resistance to one or more components of the initial drug treatment.

Most of the patients with SCCB have advanced disease, and chemotherapy presently produces only a modest improvement in survival, although long-term survival is possible. The criteria that we have described in this paper allow the selection of a subgroup of patients, whose prognosis is better than that of the majority. It is for this subgroup that further intensification of therapy is justified, with the hope of producing long-term survival from this highly malignant disease.

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Clinical Trial of Cisplatin and 5-FU Infusion as Initial Treatment for Advanced Squamous Cell Carcinoma of the Head and Neck[1]

Julie Kish,[2] Anibal Drelichman,[2] John Jacobs,[2] Judith Hoschner,[3] Jeannie Kinzie,[2] John Loh,[4] Arthur Weaver,[2] and Muhyi Al-Sarraf[2,5,*]

SUMMARY

Initial treatment with drug combinations that include cisplatin and bleomycin has been effective in reducing tumor in patients with previously untreated epidermoid cancers of the head and neck. Because of the threat of pulmonary complications from bleomycin, patients with poor pulmonary function were excluded from those studies. We conducted a trial using the combination of cisplatin (100 mg/m²) and a 96-hour infusion of 5-FU (1000 mg/m²/day) and achieved a response rate of 88.5% in 26 patients treated with two courses. Five of these patients had complete remissions and 18 had partial remissions. Nausea and vomiting (experienced by 70% of the patients) was the predominant toxic effect, and 26% experienced leukopenia. Although all patients were initially inoperable, six underwent resection following chemotherapy, and another six underwent resection after chemotherapy and irradiation. Two additional patients were treated with radiation therapy after clinically achieving complete remissions following chemotherapy, and another ten received X-ray therapy after chemotherapy. Cisplatin and 5-FU infusion are effective without being as toxic as bleomycin in patients with previously untreated advanced carcinoma of the head and neck.

[Cancer Treat Rep 66:471–474, 1982]

Initial therapy of squamous cell carcinoma of the head and neck has revolved around surgery and/or radiation therapy. Chemotherapy has usually been reserved for patients with recurrent disease (1–5). The efficacy of single agents such as methotrexate and bleomycin is well-known, but the response rate is < 50%. Cisplatin alone produced a response rate of 30%–40% (4,6). Cisplatin has most often been combined with bleomycin, which by itself has a response rate < 30% (3). The response rates of cisplatin combinations have been higher in previously untreated patients than in those who have had surgery and/or radiotherapy (7–16). Table 1 shows reported response rates achieved in previously untreated head and neck cancer when cisplatin has been employed.

The known pulmonary toxic effects associated with bleomycin preclude its use in many patients with head and neck cancer. Bleomycin toxicity is a cumulative phenomenon and is often irreversible (2). Peppard et al (11), testing both diffusion capacity and arterial blood gases, reported a 4% incidence of pulmonary toxicity.

5-FU has a response rate of 31% in patients with head and neck cancer, as previously reported (1). It has been shown that patients have less myelosuppression with continuous infusion of 5-FU than with the bolus method (17,18). In an attempt to

[1]Received Mar 2, 1981; revised Sept 24, 1981; accepted Oct 8, 1981.

[2]Departments of Oncology (*J. Kish, A. Drelichman,* and *M. Al-Sarraf*), Otolaryngology (*J. Jacobs*), Radiotherapy (*J. Kinzie*), and Surgery (*A. Weaver*), Wayne State University School of Medicine, Detroit, MI.

[3]Harper-Grace Hospitals, Detroit, MI.

[4]Veterans Administration Hospital, Detroit, MI.

[5]We thank Marie Schumacher and Rose Marie Mason for secretarial assistance.

**Reprint requests to:* Muhyi Al-Sarraf, MD, Oncology Department, Wayne State University School of Medicine, 3990 John R St, Detroit, MI 48201.

TABLE 1.—Response to drug combinations including cisplatin in patients with head and neck cancer

Authors (ref No.)	Drugs*	No. of patients	% response
Brown et al (16)	C, V, B	23	74
Elias et al (8)	C, B, M	19	84
Hong et al (9)	C, B	58	72
Randolph et al (12)	C, B	21	71
Spaulding et al (14)	C, O, B	30†	97
Weaver et al (15)	C, O, B	75	80
Wittes et al (6)	C	48	40
	C, B	33	71

*C = cisplatin, V = vinblastine, B = bleomycin, M = methotrexate, and O = vincristine.

†Most of these patients were operable. Chemotherapy was used as a preoperative adjuvant.

avoid the pulmonary toxicity of bleomycin and to maintain a high response rate, we chose a combination of cisplatin and 96-hour infusion of 5-FU for patients with previously untreated head and neck cancer. This report details our preliminary results.

MATERIALS AND METHODS

Patients with inoperable stage IV squamous cell cancers of the head and neck were included in this study. The inoperability status was based on the surgical evaluation that the primary lesion and/or the regional nodes could not be removed with cancer-free margins.

Pretreatment assessment included history and physical examination, triple endoscopy and appropriate biopsies, cbc with platelet count, multiphasic profile, 24-hour creatinine clearance, bilateral audiograms, chest X-ray, iv pyelogram, and other appropriate scans indicated to rule out distant metastases.

All patients were required to have a BUN < 20 mg/100 ml; a serum creatinine < 1.5 mg/100 ml and/or creatinine clearance ≥ 60 ml/minute; an iv pyelogram that showed at least one nonobstructed, functioning kidney; a wbc count of 4000/mm^3 and a platelet count of 100,000/mm^3; and no distant metastases. All patients had measurable disease.

Hydration with 2 liters of 5% dextrose in ½ normal saline with 40 mEq of KCl was initiated on Day 0. On Day 1, patients received 12.5 g of mannitol as an iv bolus, followed by a dose of 100 mg/m^2 of cisplatin iv and then by a 4-hour infusion of 25 g of mannitol in 1 liter of 5% dextrose in ½ normal saline with 30 mEq of KCl. Then the 5-FU infusion was started. The patient received 1000 mg/m^2/day of 5-FU in 2 liters of 5% dextrose in ½ normal saline as a 96-hour infusion (over 4 days). Allopurinol was administered at a dose of 300 mg/day. Chlorpromazine (10 mg im) was administered as needed. The second course of chemotherapy was repeated 3 weeks later or as soon as any toxic effects subsided. After two courses, patients were evaluated for operability.

Criteria for response were defined as follows: *complete response (CR)*—complete disappearance of all measurable disease and symptoms; *partial response (PR)*—a $\geq 50\%$ but $< 100\%$ reduction in the product of the diameters of all measurable lesions in the absence of new lesions; *minimum response*—a $> 25\%$ but $< 50\%$ reduction in the product of the diameters of all measurable lesions in the absence of new lesions; and *no change*—no change in disease status or less than a minimum response.

RESULTS

Twenty-six patients were treated with this combination at the Wayne State University affiliated hospitals in Detroit, MI. All patients had a histologic diagnosis of squamous cell carcinoma of the head and neck; all were clinically stage IV and inoperable; and all were evaluated by a surgeon, a radiation therapist, and a medical oncologist prior to treatment.

Twenty-four male and two female patients were treated. The median age was 54 years (range, 22–70). Morphology was moderately differentiated in 15 patients, poorly differentiated in nine, and well-differentiated in two. Fifty percent (13 patients) had multiple primaries of the head and neck or esophagus. Twenty-four patients had had no previous therapy, and two had had previous radiotherapy and had developed a second primary 2 and 7 years later.

All patients completed two courses of chemotherapy. After the second course we observed five of the 26 patients (19.2%) to be in CR and 18 (69.3%) to be in PR; three (11.5%) showed no response with either course.

Nausea and vomiting was experienced by 70% of the patients; it was mild in six, moderate in ten, and severe in three. Leukopenia occurred in 26.9% of the patients but was severe only in one who was undergoing simultaneous radiotherapy to the esophagus. Mild renal toxic effects occurred in 26% of the patients. Moderate thrombocytopenia (platelet count, 50,000–75,000/mm^3) occurred in one patient, mild stomatitis in two, mild alopecia in one, and phlebitis in two; none of the patients experienced diarrhea.

Six patients had radical resection after chemotherapy alone (two with CR and four with PR), and six had radical resection after chemotherapy and preoperative radiotherapy of 5000 rad. Twelve patients had radiotherapy only (6600 rad) after chemotherapy (two with CR, seven with PR, and three with no change). The two patients (one with CR and one with PR) with previous radiotherapy could be given no further radiotherapy but were maintained in response with chemotherapy given every 3–4 weeks.

Two patients had negative histology for tumor at the time of surgical resection after chemotherapy, and one patient had no viable tumor after chemotherapy and irradiation. One patient had negative biopsy after chemotherapy, and two patients had negative biopsies after chemotherapy plus radiotherapy.

At this analysis, all patients have had a minimum of 1 year of followup. Thirteen patients have no evidence of disease (60+ weeks); one with local and regional cancer is receiving systemic chemotherapy; three who were free from malignant disease died from other causes (aspiration pneumonia, a car accident, and a fall); and nine died with persistent disease (three who had no change after chemotherapy) or recurrent cancer (six who had PRs after chemotherapy).

DISCUSSION

Several recent innovations in the use of chemotherapy appear to form the basis for an increasing role for this modality in the treatment of advanced epidermoid cancer of the head and neck. Many combinations of agents with cisplatin have shown high response rates in previously untreated head and neck cancer (7–16). Bleomycin therapy cannot be safely utilized in some of these patients because a significant proportion develop advanced pulmonary dysfunction.

The combination of high-dose cisplatin and 5-FU infusion has demonstrated significant activity in advanced carcinoma of the head and neck without the toxicity of bleomycin. Since there are no major pulmonary toxic effects associated with this regimen, it has a broader range of applicability in this patient population comprised predominantly of heavy smokers.

The 88.5% response rate with this combination is comparable to that obtained with cisplatin and bleomycin combined (table 1). The toxic effects were mild and reversible and the combination was well-tolerated. No impairment was produced that precluded other therapies or interfered with daily activities.

Shapshay et al (19) found that the advantage to using cisplatin-containing chemotherapy initially appears to be that cisplatin accentuates the process of tumor cell differentiation; this may ultimately lead to better resectability (19), as illustrated by several patients in this series. It is unknown whether initial chemotherapy will ultimately lead to increased disease-free remission or survival. Many randomized studies are under way to answer this question.

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Influence of Renal Failure on Myelosuppressive Effects of Melphalan: Cancer and Leukemia Group B Experience [1,2]

G. G. Cornwell, III,* T. F. Pajak, O. R. McIntyre, S. Kochwa, and H. Dosik [2,3]

SUMMARY

The influence of renal insufficiency on melphalan-induced myelosuppression was examined during the initial 10 weeks of treatment in 295 patients with multiple myeloma. Patients were randomized to receive either oral melphalan (0.15 mg/kg/day for 7 days, followed by 0.05 mg/kg/day after recovery from the wbc count nadir) or iv melphalan (16 mg/m² every 2 weeks for four doses, followed by a single dose every 4 weeks). All patients received a 6-week tapering course of prednisone. Patients with renal insufficiency (BUN ≥ 30 mg/100 ml) had a significantly higher frequency of severe leukopenia (≤ 1000 cells/mm³) following iv melphalan than did patients with normal renal function (50% vs 15%, respectively; $P = 0.007$). The latter effect resulted in an increased frequency of drug-related deaths secondary to infection. The frequency of severe thrombocytopenia (≤ 25,000 cells/mm³) was also greater in patients with renal failure following iv melphalan therapy. Reduction of iv melphalan dose to 50% in patients with elevated BUN reduced the frequency of these complications to levels that were not significantly different from those observed in patients with normal renal function. The frequency of severe myelosuppression was independent of renal function in patients receiving oral melphalan. Possible explanations for these findings are discussed.

[Cancer Treat Rep 66:475–481, 1982]

Melphalan is the chemotherapeutic agent most commonly employed in the treatment of multiple myeloma. Despite its frequent use in a disease often complicated by renal failure, the influence of this complication on the metabolism and toxicity of melphalan is not well-understood.

Over the past 15 years, a large number of patients with multiple myeloma have been treated by the Cancer and Leukemia Group B (CALGB) with oral melphalan in various schedules. Renal failure has not significantly increased the incidence of severe myelotoxicity in these patients. Iv melphalan was

[1]Received Apr 14, 1981; revised Sept 13, 1981; accepted Oct 5, 1981.

[2]This study was conducted by the Cancer and Leukemia Group B (CALGB) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following institutions participated in the study: Dartmouth-Hitchcock Medical Center, Hanover, NH (*G.G. Cornwell, III*, and *O.R. McIntyre*, CA-04326); CALGB Operations Office, Scarsdale, NY (*T.F. Pajak*, CA-16118); Mount Sinai Medical Center, New York, NY (*S. Kochwa* and *J. Cuttner*, CA-04457); Jewish Hospital and Medical Center of Brooklyn, NY (*H. Dosik*, CA-25119); Bowman Gray School of Medicine, Winston-Salem, NC (*M.R. Cooper*, CA-03927); Roswell Park Memorial Institute, Buffalo, NY (*E.S. Henderson*, CA-02599); Thomas Jefferson University Hospital, Philadelphia, PA (*F.I. Haurani*); Mayo Clinic, Rochester, MN (*R.A. Kyle*, CA-04646); Medical College of Virginia, Richmond (*M.J. Flaherty*); West Virginia University Hospital, Morgantown (*S.V. Ramanan*); University of Pretoria, Republic of South Africa (*G. Falkson*); Long Island Jewish Medical Center, New Hyde Park, NY (*D. Prager*, CA-11028); Massachusetts General Hospital, Boston (*M. Greenberg*, CA-12449); Columbia-Presbyterian Hospital, New York, NY (*R.R. Ellison*, CA-12011); University of Missouri, Columbia (*P. Henry*, CA-12046); Upstate Medical Center of Syracuse, NY (*A.J. Gottlieb*, CA-21060); and Methodist Hospital of Brooklyn-SUNY (*J.M. Schwartz*, CA-25119).

[3]We appreciate the expert technical assistance of Linda P. Glowienka, Kim Seaver, and Janice Davis.

**Reprint requests to:* Gibbons G. Cornwell, III, MD, Department of Medicine, Dartmouth-Hitchcock Medical Center, Hanover, NH 03755.

first used to treat myeloma in 1971. Patients received the first six courses of iv melphalan (16 mg/m²) at intervals of 2 weeks. An initial 10-week tapering course of prednisone was also given. Subsequent doses of melphalan were given at 4-week intervals followed by a 7-day course of prednisone. In 47 patients treated with this regimen, 35% developed severe leukopenia (≤ 1000 cells/mm³), 30% had life-threatening infection, and 13% died of infection related to leukopenia (1). Since the patients receiving iv melphalan appeared to survive longer than those receiving oral melphalan (median survival: 36 vs 16 months, respectively; $P = 0.16$) (2), a subsequent CALGB protocol, activated in July 1974, was modified to allow further comparison of oral and iv melphalan in untreated myeloma patients. In view of previously noted toxicity, the initial iv melphalan therapy was reduced from six to four doses at 2-week intervals prior to monthly treatment courses. The results of this melphalan schedule and the frequency of severe leukopenia, death related to infection, and severe thrombocytopenia are reported.

MATERIALS AND METHODS

Treatment Groups

A total of 310 previously untreated patients with multiple myeloma were randomized to receive oral melphalan (0.15 mg/kg/day for 7 days, with a maintenance dose of 0.05 mg/kg/day after recovery from peripheral wbc count nadir) or iv melphalan (16 mg/m² every 2 weeks for 6 weeks, then every 4 weeks). The total oral melphalan dose for a 70-kg (1.7-m²) patient during the first 10 weeks of treatment was approximately 220 mg, whereas the total iv dose was 110 mg. All patients received a 6-week tapering course of prednisone starting at 0.8 mg/kg/day. Informed consent was obtained prior to entry in the study. Two other treatment programs in this study, ie, those with carmustine (BCNU) or lomustine (CCNU), are not discussed in this report. In view of the extensive experience of CALGB with oral melphalan in this dose schedule, the randomization procedure was designed so that twice as many patients received iv melphalan as oral melphalan.

At the initiation of the study, adjustment of oral and iv melphalan doses was made on the basis of total leukocyte and platelet counts (tables 1 and 2). No adjustment was made for patients with an elevated BUN or serum creatinine. After the first year of this study, sufficient evidence had accumulated to indicate a significant increase in severe leukopenia following iv melphalan treatment in patients with renal failure (BUN ≥ 30 mg/100 ml). Consequently, the study was amended to require a 50% reduction in the dose of iv melphalan in patients with BUN ≥ 30 mg/100 ml.

TABLE 1.—Dose modifications of oral melphalan for initial and subsequent doses

Wbcs/mm³	Platelets/mm³	% of full dose
≥ 4000	≥ 100,000	100
≥ 3000	≥ 75,000	75
≥ 2000	≥ 50,000	50
< 2000	< 50,000	0

Drug Formulation and Administration of Iv Melphalan

Melphalan powder (Burroughs Wellcome Co, Research Triangle Park, NC) was provided by the National Cancer Institute (Bethesda, MD) and stored at –4°C until use. Aliquots (100 mg) of powder were dissolved in 1 ml of 10% ethanol containing K_2HPO_4, propylene glycol, and HCl (final pH 7) prior to dilution in normal saline to a final concentration of 10 mg/ml. The appropriate dose was injected directly iv or into the tubing of a running iv.

RESULTS

Of 310 patients randomized, 295 were considered to be evaluable for this analysis. Eight patients were ineligible for the protocol, three were disqualified for protocol violation, three were disqualified because of incomplete data, and one was improperly randomized.

Iv Melphalan Schedule

At the time of an interim analysis for the first 77 evaluable patients treated with the iv melphalan schedule (pre-addendum), high frequencies of severe leukopenia (≤ 1000 cells/mm³) (22%), drug-related deaths (10%), and severe thrombocytopenia (≤ 25,000 cells/mm³) (10%) were found, which were quite similar to those observed in the previous 1971 protocol. Patients with BUN ≥ 30 mg/100 ml during the initial 10 weeks of treatment (the period of most intensive treatment) had a significantly higher frequency of severe leukopenia (50%) than did patients with normal renal function (15%) ($P = 0.007$) (table 3). The incidence of drug-related deaths and severe thrombocytopenia was also greater in patients with renal failure ($P = 0.09$) (tables 4 and 5). There was a direct relationship between the actual elevated

TABLE 2.—Dose modifications of iv melphalan for initial and subsequent doses

Counts for initial dose		% of full dose given	Counts for subsequent doses				% of full dose given
			At time of previous dose		Nadir after previous dose		
Wbcs/mm³	Platelets/mm³		Wbcs/mm³	Platelets/mm³	Wbcs/mm³	Platelets/mm³	
≥ 5000	≥ 100,000	100*	≥ 5000	≥ 100,000	≥ 3000	≥ 75,000	100
≥ 3000	≥ 75,000	50†			> 1000	≥ 50,000	50
> 1000	< 75,000	25			≤ 1000	< 50,000	25
≤ 1000	—	0					
			≥ 3000	≥ 75,000	> 1000	≥ 50,000	50
					≤ 1000	< 50,000	25
			> 1000	< 75,000	—	—	25
			≤ 1000	—	—	—	0

*If initial dose was 16 mg/m² (100%) and nadir wbc count was ≥ 5000/mm³ or nadir platelet count was ≥ 100,000/mm³, subsequent dose was increased to 20 mg/m².

†If initial dose was 8 mg/m² (50%) and there was no further depression of blood cell counts, the second dose was 16 mg/m² (100%), provided wbc count was ≥ 5000/mm³ and platelet count was ≥ 100,000/mm³. The third dose was further escalated if the conditions in above footnote were met.

TABLE 3.—Effect of renal failure on frequency of severe leukopenia (≤ 1000 cells/mm³) during initial 10 wks of treatment*

Type of analysis	Melphalan route	BUN < 30 mg/100 ml			BUN ≥ 30 mg/100 ml			
		No. of patients	No. of patients with ≤ 1000 wbcs/mm³ (%)		No. of patients	No. of patients with ≤ 1000 wbcs/mm³ (%)		
All patients included	Iv						$P = 0.007$	
	Pre-addendum	61	9 (15)		16	8 (50)	$P = 0.01$	$P = 0.004$
	Post-addendum	90	11 (12)	$P = 0.01$	28	3 (11)	NS	
	Oral	67	1 (2)		33	3 (9)		
Adjusted for deviation from protocol	Iv						$P = 0.02$	
	Pre-addendum	61	6 (10)		16	6 (38)	$P = 0.03$	$P = 0.005$
	Post-addendum	90	4 (4)	$P = 0.07$	28	0 (0)	NS	
	Oral	67	0 (0)		33	1 (3)		

*NS = not significant.

BUN concentration and the frequency of severe myelotoxicity, but the total number of patients with severe renal failure was too small to permit statistical analysis.

An additional 118 patients received iv melphalan following the decrease in dose for patients with renal failure (post-addendum). The frequency of severe leukopenia in patients with BUN ≥ 30 mg/100 ml was 11%, a significant reduction from the pre-addendum level of toxicity ($P = 0.01$). This value was not significantly different from that observed in patients with normal renal function who received iv melphalan (table 3). The frequency of severe thrombocytopenia was not significantly altered by the addendum (table 5); consequently, the records of all patients who developed severe myelotoxicity following iv or oral melphalan were re-evaluated to identify any possible deviations from dose adjustments that were mandated by the protocol in the pre-addendum and post-addendum periods. Twenty patients were identified who could be included in one of the following categories: (*a*) the dose of melphalan was not appropriately adjusted for leukopenia or thrombocytopenia in accordance with tables 1 and 2, (*b*) melphalan was given together with radiation therapy or before the nadir leukocyte count following radiation therapy, (*c*) the initial dose of iv melphalan was not appropriately adjusted for renal failure in the post-addendum period, or (*d*) an excessive dose of melphalan was erroneously given (tables 6 and 7). A separate ("adjusted") statistical analysis was then performed following the exclusion of patients in these categories.

The adjusted analysis indicated that patients with

TABLE 4.—Frequency of drug-related deaths in patients receiving melphalan*

Type of analysis	Melphalan route	BUN < 30 mg/100 ml: No. of patients	BUN < 30 mg/100 ml: No. of drug deaths (%)	BUN ≥ 30 mg/100 ml: No. of patients	BUN ≥ 30 mg/100 ml: No. of drug deaths (%)
All patients included	Iv				
	Pre-addendum	61	4 (7)	16	4 (25)
	Post-addendum	90	1 (1)	28	2 (7)
	Oral	67	0 (0)	33	1 (3)
			P = 0.09; NS		NS; NS; P = 0.06
Adjusted for deviation from protocol	Iv				
	Pre-addendum	61	1 (2)	16	3 (19)
	Post-addendum	90	1 (1)	28	0 (0)
	Oral	67	0 (0)	33	0 (0)
			P = 0.03; NS		P = 0.08; NS; P = 0.05

*NS = not significant.

TABLE 5.—Effect of renal failure on frequency of severe thrombocytopenia (≤ 25,000/mm³) during initial 10 wks of treatment*

Type of analysis	Melphalan route	BUN < 30 mg/100 ml: No. of patients	BUN < 30 mg/100 ml: No. of patients with ≤ 25,000 platelets/mm³ (%)	BUN ≥ 30 mg/100 ml: No. of patients	BUN ≥ 30 mg/100 ml: No. of patients with ≤ 25,000 platelets/mm³ (%)
All patients included	Iv				
	Pre-addendum	61	4 (7)	16	4 (25)
	Post-addendum	90	3 (3)	28	5 (18)
	Oral	67	1 (2)	33	0 (0)
			P = 0.09; NS		NS; P = 0.04; P = 0.01
Adjusted for deviation from protocol	Iv				
	Pre-addendum	61	1 (2)	16	3 (19)
	Post-addendum	90	1 (1)	28	0 (0)
	Oral	67	0 (0)	33	0 (0)
			P = 0.03; NS		P = 0.03; NS; P = 0.05

*NS = not significant.

renal failure receiving iv melphalan had a significantly higher incidence of severe leukopenia during the pre-addendum period as compared to patients with normal renal function ($P = 0.02$) and that the frequencies of drug-related deaths ($P = 0.03$) and severe thrombocytopenia ($P = 0.03$) were also significantly higher in the former group. In addition, a significant reduction in severe leukopenia (38% vs 0%; $P = 0.003$) and severe thrombocytopenia (19% vs 0%; $P = 0.03$) occurred in the post-addendum period for azotemic patients receiving iv melphalan (tables 3 and 5). The incidence of drug-related deaths among patients with renal failure also decreased following the dose modification ($P = 0.08$)(table 4).

Oral Melphalan Schedule

Among 100 evaluable patients receiving the oral melphalan schedule, 33% had evidence of renal insufficiency on entry in the study. There was no evidence that the frequency of severe leukopenia, drug-related deaths, or severe thrombocytopenia was significantly increased in the patients with renal insufficiency as compared to those with normal renal function. In the adjusted analysis, patients with renal failure had a significantly lower incidence of these complications when compared to patients receiving iv melphalan in the pre-addendum period, but there was no difference from patients receiving iv melphalan in the post-addendum period (tables 3–5).

Patient Response and Survival

Despite the greater degree of myelosuppression in patients receiving iv melphalan, analysis of response and survival does not currently show any advantage of this route over oral melphalan. The response frequencies for patients with BUN ≥ 30

TABLE 6.—Patients with normal renal function who developed severe leukopenia (≤ 1000 cells/mm³) and/or severe thrombocytopenia (≤ 25,000 cells/mm³) during initial 10 wks of therapy

Patient No.	Melphalan route*	Wbc count (cells × 10^3/mm³)		Day of nadir	Platelet count (cells × 10^3/mm³)		Day of nadir	Comments†
		Initial	Nadir		Initial	Nadir		
1	Iv pre	2.5	0.1	22	170	4	19	1
2	Iv pre	3.6	0.3	8	199	3	15	2,4
3	Iv pre	3.5	0.3	35	225	21	35	3,4
4	Iv pre	6.1	0.2	10				4
5	Iv pre	10.0	0.7	15	265	8	15	
6	Iv pre	6.3	1.0	37				
7	Iv pre	5.1	0.9	23				
8	Iv pre	7.2	1.0	29				
9	Iv pre	5.1	1.0	15				
10	Iv post	4.0	0.3	29	167	20	27	1
11	Iv post	3.8	0.4	29	200	19	31	2
12	Iv post	3.0	0.9	56				1
13	Iv post	6.3	0.9	45				1
14	Iv post	3.8	0.7	30				1
15	Iv post	6.4	0.6	27				3,4
16	Iv post	3.3	0.5	42				3,4
17	Iv post	5.9	0.2	33				4
18	Iv post	2.5	0.4	13	35	12	13	
19	Iv post	5.6	0.7	16				
20	Iv post	5.2	0.5	18				
21	Oral				15	8	31	1
22	Oral	5.7	0.2	33				

*Pre = pre-addendum; post = post-addendum.

†1 = dose of melphalan was not adequately adjusted for leukopenia; 2 = melphalan was given before nadir leukocyte count following radiation therapy; 3 = excessive dose of melphalan was erroneously given; and 4 = death was related to leukopenia.

TABLE 7.—Patients with abnormal renal function who developed severe leukopenia (≤ 1000 cells/mm³) and/or severe thrombocytopenia (≤ 25,000 cells/mm³) during initial 10 wks of therapy

Patient No.	Melphalan route*	Wbc count (cells × 10^3/mm³)		Day of nadir	Platelet count (cells × 10^3/mm³)		Day of nadir	Comments†
		Initial	Nadir		Initial	Nadir		
1	Iv pre	4.2	0.8	17	110	6	17	1,5
2	Iv pre	5.2	0.8	29	212	20	29	5
3	Iv pre	6.0	0.6	27				1
4	Iv pre	8.2	0.3	21				5
5	Iv pre	7.6	0.3	14				5
6	Iv pre	6.5	0.8	11				5
7	Iv pre	5.8	0.6	16	148	8	15	
8	Iv pre	12.4	0.7	21	155	17	17	
9	Iv post	11.9	0.9	13	216	20	13	4,5
10	Iv post	7.8	0.2	28	295	11	28	4,5
11	Iv post	7.1	0.7	14				3
12	Iv post				250	10	57	1
13	Iv post				210	12	19	4
14	Iv post				180	16	14	4
15	Oral	4.6	0.8	42				1
16	Oral	8.7	0.9	18				2
17	Oral	3.4	0.6	47				

*Pre = pre-addendum; post = post-addendum.

†1 = dose of melphalan was not adequately adjusted for leukopenia; 2 = melphalan was given before nadir leukocyte count following radiation therapy; 3 = renal failure developed shortly after initial dose of melphalan; 4 = initial dose of iv melphalan was not adjusted for renal failure; and 5 = death was related to leukopenia.

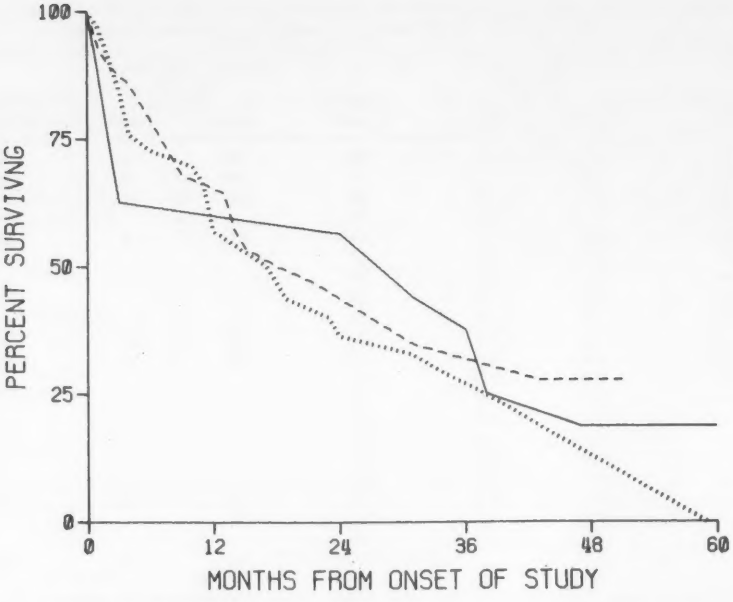

FIGURE 1.—% survival of patients with elevated BUN (≥ 30 mg/100 ml) following treatment with pre-addendum (——) or post-addendum (- - -) iv melphalan or oral melphalan (· · ·).

mg/100 ml were 61% for those receiving oral melphalan and 56% (pre-addendum) and 41% (post-addendum) for those receiving iv melphalan ($P > 0.2$). The survival results for these three groups were also very similar (fig 1).

Among azotemic patients there was no statistical long-term survival advantage associated with receiving the pre-addendum iv dose as compared to the oral dose.

DISCUSSION

Studies by Alberts et al (3) have shown that approximately 13% of iv melphalan is excreted in the 24-hour urine of cancer patients with normal renal function. Subsequent studies in dogs have shown that renal clearance of iv melphalan is significantly decreased in the presence of renal failure and that marked increase in bone marrow toxicity results (4). Although plasma and urine concentrations of melphalan were not measured in the patients reported in the present study, the increased myelosuppressive effects of iv melphalan in patients with renal failure suggest that renal clearance of the drug was impaired.

The failure of renal insufficiency to enhance the myelotoxic effects of oral melphalan may be related to lower blood levels of melphalan achieved with oral administration rather than to intrinsic differences in the two forms of the drug. Alberts et al (5), studying plasma concentrations of melphalan and percent of dose excreted in the urine following equivalent doses of oral and iv melphalan, have found significantly higher concentrations in patients receiving the iv form of the drug. In addition, peak plasma levels of alkylating agents are known to be related to bone marrow toxicity. It would be anticipated, therefore, that intermittent iv melphalan would be associated with greater potential for myelotoxicity, especially in the presence of renal failure, than daily oral melphalan. The present study indicated in fact that twice as much oral melphalan could be tolerated without significant toxicity in the first 10 weeks of treatment when compared to the iv drug.

It has been shown that gastrointestinal absorption of oral melphalan in man is variable and unpredictable (5). This observation has potential implications for patients with myeloma who fail to respond to oral melphalan. In view of this observation and the influence of renal insufficiency on

plasma kinetics, it is clear that future studies of oral and iv melphalan will require measurements of plasma drug concentrations and clearance as well as appropriate dose augmentation and reduction.

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Cardiac Abnormalities After AMSA Administration[1,2,3]

Laurel J. Steinherz,* Peter G. Steinherz, Deborah Mangiacasale, Charlotte Tan, and Denis R. Miller[4,5]

SUMMARY

AMSA produced changes on echocardiograms of 18 of 27 patients. Seven patients developed clinical congestive heart failure. The occurrence of cardiac abnormalities was influenced by the previous anthracycline dose and the rate and dose of AMSA administered. No changes were seen in six patients, with a total anthracycline dose ≤ 400 mg/m² and < 200 mg/m² of AMSA given on 2 consecutive days. Seven of eight patients had abnormalities with an anthracycline dose ≤ 400 mg/m² but ≥ 200 mg/m² of AMSA in 48 hours. Nine of 11 patients had abnormalities when AMSA was given after a total anthracycline dose of 400 mg/m². When the total combined anthracycline and AMSA dose was ≥ 900 mg/m², 13 of 14 patients had abnormalities while only three of 11 who received less than this dose had abnormalities. The abnormalities may be reversible with prompt treatment and discontinuation of drug.

[Cancer Treat Rep 66:483–488, 1982]

AMSA is a new acridine derivative which is active against a number of experimental tumor systems (1). Phase I and II clinical trials revealed responses in patients with malignancy. Myelosuppression is its major toxic effect; other side effects include nausea, emesis, mucositis, diarrhea, malaise, alopecia, and abnormalities of liver function (1–3). No cardiotoxicity was noted in the early studies. Eight documented cases of arrhythmia during or immediately after AMSA infusion have now been reported in > 1500 patients treated (4–6).[6] This prospective study identified reversible abnormalities on serial echocardiograms of 18 patients (7) and clinical congestive heart failure (CHF) in seven children receiving AMSA who had received previous anthracyclines with or without exposure to other potentially cardiotoxic treatment.

MATERIALS AND METHODS

Patients

Patients with various malignancies followed by the Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, who had relapsed during conventional therapies and who had no other efficacious reported treatment available to them, were entered in phase I and II chemotherapy trials after informed consent. Twenty-seven patients given AMSA were studied. Twelve had acute lymphoblastic leukemia, seven had osteogenic sarcoma, three had non-Hodgkin's lymphoma, two had rhabdomyosarcoma, and one each had Wilms' tumor, chondrosarcoma, and acute nonlymphoblastic leukemia. There were 19 males and eight females; median age was 10 years (range, 1–24).

[1]Received Apr 14, 1981; revised July 28, 1981; accepted Aug 7, 1981.

[2]Supported in part by Public Health Service grants CA-23742 and CA-05826 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Presented in part at the 71st Annual Meeting of the American Association for Cancer Research, San Diego, CA, May 29, 1980.

[4]Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, New York, NY.

[5]We thank Ms. Mary Garone and Ms. Lee Cascio for secretarial assistance.

[6]Department of Health, Education and Welfare. Urgent NCI update on *m*-AMSA cardiotoxicity. July 29, 1980.

**Reprint requests to:* Laurel J. Steinherz, MD, Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

All of the patients had received anthracyclines (doxorubicin, daunorubicin, or zorubicin) prior to AMSA administration. Cardiac abnormalities were demonstrated in two patients before AMSA administration. They were given low doses and had no further changes. The cumulative anthracycline dose was < 400 mg/m² in 14 patients, but eight had reached their limit of 450–500 mg/m². Five patients had received mediastinal radiation therapy (table 1). Seven other patients had radiation to the spine, lung, or sternum, with some scatter to the heart. Three patients had a previous course of deoxycoformycin.

AMSA Administration

Initially, weekly iv single doses of AMSA were escalated from 30 to 70 mg/m². This was followed by a twice weekly and then a daily schedule. During the phase II study patients with leukemia received a dose of 100–150 mg/m²/day for 3–5 days and children with solid tumors received a dose of 40–100 mg/m²/day for 1–3 days. Courses were repeated after toxic effects had cleared (2–3 weeks). AMSA was dissolved in N,N-dimethylacetamide and lactic acid, added to 150–250 ml of 5% dextrose in water, and infused over a period of *1 hour* to reduce the incidence of phlebitis.

Methods

M-mode echocardiograms were obtained with a Picker 80C Echoview System and recorded on a Hofrell Ultraviolet Strip Chart Recorder. In general, a 3.5-MHz transducer was used, supplemented on rare occasions by use of a 2.25-MHz transducer.

Left ventricular internal dimension and function parameters were measured from the left ventricular tracing at a paper speed of 50 mm/second. The efficiency of contraction was measured by obtaining the fractional shortening, ie, the percent that the end diastolic internal dimension (the maximal distance between the posterior wall and the septum) is decreased with contraction (normally 30%–45%). This can be extrapolated to three dimensions (ejection fraction) by cubing the measurements. The ejection fraction thus obtained (normally 68%–80%) is comparable to that obtained by cardiac catheterization and cineangiography (5).

Systolic time intervals were measured from the tracings of the aortic and pulmonary valves at a paper speed of 100 mm/second. The systolic time intervals compare the pre-ejection period (PEP), ie, the time required by the left ventricle to generate sufficient pressure to open the semilunar valve, to the ejection time (ET), ie, the length of time the valve remains open. The less efficient the ventricle the longer it will take to open the valve and the shorter the time it can be maintained open. Thus, the PEP/ET ratio increases with poor contractility. Normal values for our laboratory depend on the age of the patient but are generally < 0.40 (8).

Echocardiograms were initially obtained only after AMSA administration. After the first few patients with abnormalities were identified, each new patient in the study was evaluated prior to every AMSA course and after the last dose of the drug. When there were significant abnormalities additional echocardiograms were performed. Cardiac monitoring during infusions was not performed. Clinical evaluation was performed by the pediatric cardiologist when clinical cardiovascular problems occurred.

RESULTS

Eighteen of the 27 patients had one or more cardiac abnormalities demonstrated by echocardiography after AMSA therapy (table 2). Sixteen patients had an increase in their left ventricular PEP with respect to ET. The PEP/ET ratio increased by 19%–133% of baseline (average, 46%). Nine patients had a decrease in fractional shortening compared to the baseline value by an average of 33%.

Two of the 18 patients with abnormalities had pericardial involvement by the malignant process and are not evaluable for AMSA cardiotoxicity. The distribution of the remaining 16 abnormalities in the 25 patients was related to the previous dose of anthracyclines administered and the rate and total dose of AMSA administration (table 1). The total previous anthracycline dose was calculated by adding the doses of doxorubicin, daunorubicin, and 4′-epiadriamycin, and one fifth of the zorubicin dose together. The six patients who had < 400 mg/m² of previous anthracyclines and < 200 mg/m² of AMSA given on 2 consecutive days showed no abnormalities. Seven of the eight patients showed abnormalities with anthracycline doses < 400 mg/m² who were given ≥ 200 mg/m² of AMSA over 2 consecutive days. Six of eight patients with anthracycline doses > 400 mg/m² and ≤ 200 mg/m² of AMSA had changes on echocardiograms, while all three patients with anthracycline doses > 400 mg/m² and ≥ 200 mg/m² of AMSA had abnormalities. Of 14 patients for whom the sum of the total anthracycline dose plus the total AMSA dose was ≥ 900 mg/m², 13 had abnormalities. Five of the six patients with clinical CHF were in this group. Only three of 11 patients with total doses < 900 mg/m² showed abnormalities, and two

TABLE 1.—Cardiac changes with AMSA*

Treatment group (total previous anthracycline + 2-day AMSA dose) in mg/m²	Previous therapy			AMSA (mg/m²)			Changes§		Risk index¶
	Total anthracycline dose (mg/m²)†	Radiation therapy‡	Deoxyco-formycin	2 days	Within 1 wk	Total	Echocar-diogram	CHF	
≤ 400 + < 200	136	–	–	150	225	225	N	–	361
	180	4000M	–	75	150	150	NC	–	330
	220	–	–	70	210	560	N	–	780
	250	–	–	150	225	435	N	–	685
	345	2000M	–	70	140	420	NC	–	765
	360	–	–	150	230	230	N	–	590
≤ 400 + ≥ 200	107	–	–	240	600	1510	1,2,3	+	1610
	120	–	–	200	300	800	1,3	+	920
	148	1000M	+	200	400	400	1	–	548
	160	–	+	200	500	500	1,2	+	660
	195	–	+	200	500	500	N	–	695
	240	2400S	–	200	300	500	1,3	–	740
	252	–	–	200	500	800	1,2,3	+	1052
	300	3000S	–	200	300	650	1	–	950
> 400 + < 200	423	2100S	–	140	210	1540	1,2	–	1963
	425	–	–	150	225	500	N	–	925
	440	2700M	–	170	228	1312	1,2	–	1752
	450	–	–	150	225	450	1	–	900
	495	–	–	175	175	344	N	–	840
	500	4600M	–	120	180	483	1,2,3	–	983
	507	–	–	150	225	450	1,2,3	+	957
	521	–	–	60	120	420	1	+	941
> 400 + ≥ 200	455	1600S	–	200	400	600	1,2	–	1055
	510	1200S	–	200	300	1625	1	–	2135
	540	2000S	–	200	300	975	1,2	–	1515

*2 patients with pericardial malignancy are not included.
†*See* text.
‡Rads to mediastinum (M), spine (S).
§N = normal; NC = no change; 1 = increased pre-ejection period/ejection time; 2 = decreased fractional shortening; 3 = pericardial effusion.
¶Total anthracycline + total AMSA dose.

TABLE 2.—Cardiac changes after AMSA administration (27 patients)

Cardiac changes	No. of patients
Clinical CHF	7*
Echocardiography	18
Increased PEP/ET	16
Decreased fractional shortening	9
Pericardial effusion	6*

*1 patient with pericardial effusion and CHF and 1 with effusion alone had pericardial involvement by the malignant process.

TABLE 3.—Cardiac abnormalities with AMSA therapy

Treatment	No. of patients with abnormalities/No. of patients in treatment group
Total anthracycline dose ≤ 400 mg/m², 48-hr AMSA dose < 200 mg/m²	0/6
Total anthracycline dose ≤ 400 mg/m², 48-hr AMSA dose ≥ 200 mg/m²	7/8
Total anthracycline dose > 400 mg/m², 48-hr AMSA dose < 200 mg/m²	6/8
Total anthracycline dose > 400 mg/m², 48-hr AMSA dose ≥ 200 mg/m²	3/3
Total anthracycline + AMSA dose < 900 mg/m²	3/11
Total anthracycline + AMSA dose ≥ 900 mg/m²	13/14

of these patients had had prior deoxycoformycin (table 3).

Seven patients had clinical CHF (tachycardia, hepatomegaly, edema); of these patients one had pericardial involvement by lymphoma, and two had received high doses of anthracyclines (507 and 521 mg/m²). In four patients with minimal prior anthracycline therapy (107, 120, 160, and 252 mg/m²), the CHF was related to AMSA administration (12, 16, 18, and 27 days after the drug). The patient for whom data are shown in figure 1 had only 107 mg/m² of daunorubicin prior to AMSA. After 120 mg/m²/day of AMSA for 5 consecutive days he had pericardial effusion, and 27 days after AMSA administration he progressed to CHF. The symptoms were controlled by digitalis and diuretics. A second course of the drug with 100 mg/m²/day for 3 days resulted in further deterioration of the PEP/ET. Bone marrow remission was achieved and the AMSA dose was decreased to a maintenance schedule of 50 mg/m² administered weekly. With this regimen the PEP/ET improved and stabilized but remained in the abnormal range. The patient was maintained in remission for 3 months. After bone marrow relapse

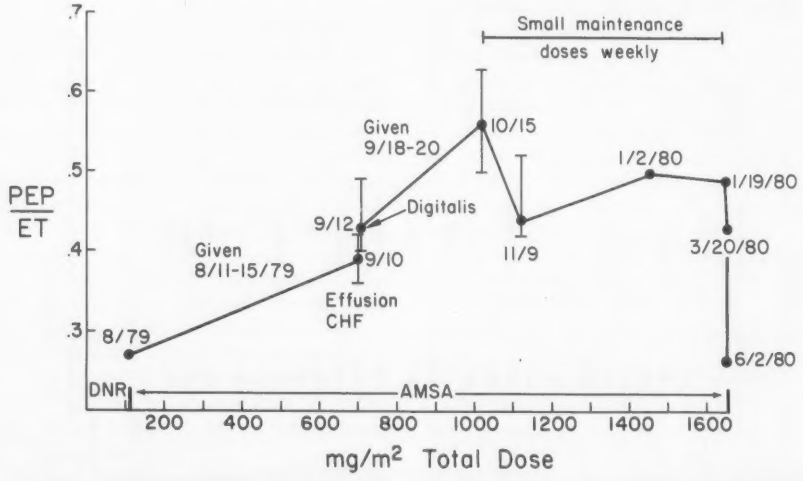

FIGURE 1.—Change in PEP/ET ratio with AMSA administration in 1 patient with acute lymphoblastic leukemia (normal for his age: < 0.35). After a dose of 107 mg/m² of daunorubicin (DNR) he is normal. 600 mg/m² of AMSA over 5 days results in pericardial effusion and CHF. PEP/ET further deteriorates with an additional 300 mg/m² of AMSA over 3 days, despite digitalization. With small weekly doses there is some improvement. Drug discontinuation results in PEP/ET return towards normal.

the AMSA was discontinued and the PEP/ET returned towards normal. The digitalis was discontinued without return of CHF.

The patients with CHF were treated with digitalis and furosemide with or without dopamine. The clinical symptoms resolved in two patients and improved in one. Four patients died with intractable CHF, including the one patient with pericardial effusion due to malignancy (with significant fluid contributing to her death). The other patients with both CHF and effusion had a small amount of fluid that was believed not to contribute to the CHF.

In all patients the hemoglobin value was maintained at > 10 g/dl. Transient episodes of severe anemia or thrombocytopenia were not associated with the appearance of cardiac abnormalities. Rise in PEP/ET ratio did not correlate with increases in blood pressure or peripheral resistance. In the one patient with cardiac failure catheterized, the peripheral resistance was below normal, as was the left ventricular stroke work and cardiac output.

DISCUSSION

AMSA is a promising new agent undergoing clinical trials. It inhibits DNA synthesis by intercalating into the double strands (1,9). It can form free radicals. This mechanism is similar to that of the anthracyclines. Some cardiotoxicity, therefore, may be anticipated. Cardiac abnormalities were not mentioned in the animal or early clinical studies. However, there is no description of how the patients were followed. Without careful monitoring of the seriously ill patients some of the cardiac effects could be missed or ascribed to other causes. We had similar difficulties initially. Many of the patients were critically ill with many organ abnormalities and were receiving therapies directed at correcting those abnormalities. Some patients had infections and were receiving multiple antibiotics, blood component replacement, and/or, at times, parenteral nutrition. Under these conditions it is sometimes difficult to establish clear cause and effect relationships to any one therapy. In patients with high doses of previous anthracycline therapy the development of CHF may have been late-onset anthracycline cardiotoxicity, a marginally compensated cardiac function decompensated by the strains of serious illness, or the tipping of a delicate balance by AMSA administration. In patients with low previous doses of other cardiotoxic agents the cardiac decompensation was much more clearly related to AMSA administration.

The occurrence of cardiac changes was influenced by the previous anthracycline dose and the rate and dose of AMSA administered. In patients with < 400 mg/m^2 of previous anthracyclines, low doses of AMSA (< 200 mg/m^2 in 48 hours) produced no cardiac abnormalities, while seven of eight patients showed changes when ≥ 200 mg/m^2 was given in 48 hours. Previous exposure to > 400 mg/m^2 of anthracyclines made the rate of AMSA administration less important. Nine of 11 patients showed abnormalities. In at least one patient, the changes, unlike anthracycline cardiomyopathy, were quickly reversible with drug discontinuation. The observed changes were similar to those we reported with cyclophosphamide (10). We did not observe the arrhythmias reported (4–7). Although the patients were not monitored during AMSA infusion, ventricular fibrillation, if present, would have been observed.

A risk index for cardiac changes could be constructed by adding the total anthracycline dose to the total AMSA dose administered. Thirteen of 14 patients with a dose ≥ 900 mg/m^2 showed abnormalities. Five of the six patients with clinical CHF were in this group. Only three of 11 patients with a dose < 900 mg/m^2 had abnormalities: two of these three patients had previously received deoxycoformycin, a drug that is also cardiotoxic, and the third patient had received 2400 rads to the neuraxis, with some radiation to the posterior aspect of the heart.

AMSA is metabolized in the liver into a thioether of acridine. It is not known whether the cardiac toxicity is due to AMSA, to one of its metabolites (11), or to the vehicle used for reconstitution of the drug. AMSA is suspended in *N,N*-dimethylacetamide and lactic acid. Vehicle control studies have shown a contribution to liver, lymphoid, and gastrointestinal lesions by the vehicle itself (12). No mention of cardiotoxicity is made in the animal studies.

In conclusion, cardiac changes can follow AMSA administration. These abnormalities may be reversible with prompt treatment and discontinuation of drug. Patients with > 400 mg/m^2 of previous anthracyclines, those who receive > 200 mg of AMSA within 48 hours, and those who have a total dose of anthracycline plus AMSA ≥ 900 mg/m^2 are at highest risk. In future studies, patients receiving doses ≥ 200 mg/m^2 of AMSA over 48 hours should have cardiac function monitored carefully and the drug doses modified accordingly. The total dose of AMSA plus anthracyclines should exceed 900 mg/m^2 only if normal cardiac status has been confirmed by echocardiography and radionuclide angiography before each new course.

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Combined Radical Radiation Therapy and Chemotherapy for Primary Squamous Cell Carcinoma of the Anal Canal [1]

B. J. Cummings,* W. D. Rider, A. R. Harwood, T. J. Keane, G. M. Thomas, C. Erlichman, and S. Fine [2,3]

SUMMARY

Radical radiation therapy (5000 rads in 20 fractions in 4 weeks) combined with iv mitomycin (10 mg/m²) and 5-FU (1000 mg/m²/24 hours for 4 days) was used to treat 13 patients with locally advanced but operable squamous cell carcinoma of the anal canal. All patients achieved local control and retained anal continence, and none developed metastases. The patients were followed from 4 to 34 months (median, 12). Severe acute gastrointestinal toxic effects were seen in three patients; the same patients had significant thrombocytopenia or leukopenia. Treatment with this combined program may allow conservative management of squamous cell carcinoma of the anal canal and should be considered as an alternative to abdominoperineal resection.

[Cancer Treat Rep 66:489–492, 1982]

Complete regression of primary squamous cell carcinoma of the anal canal following treatment with the combination of radiation therapy, mitomycin, and 5-FU has been reported both in patients treated preoperatively (1–3) and in patients treated with radical radiation and chemotherapy without surgery (4). This report describes our further experience with the combination of radical radiation therapy and chemotherapy, confirming the initial impression of satisfactory local control of these carcinomas by this treatment program, and discusses the acute toxic effects that occur in some patients.

MATERIALS AND METHODS

Thirteen patients with previously untreated operable squamous cell carcinoma of the anal canal were treated with combined radiation therapy and cytotoxic chemotherapy between May 1978 and December 1980. One additional patient seen during this period who was eligible for combined treatment received radiation therapy alone by physician preference. Patients with distant metastases, clinically or radiologically demonstrated lymph node metastases, or recurrence after previous attempted curative resection were excluded from the study. Patients whose carcinoma had destroyed the anal sphincter mechanism so that they were incontinent were also excluded.

All 13 patients had biopsy-proven squamous cell carcinoma of the anal canal and had each been assessed as eligible for abdominoperineal resection, but not for local resection of the carcinoma with preservation of the anal sphincter. There were nine females (30–65 years of age) and four males (25–82 years of age). The patients were staged according to the criteria of the Union Internationale Contre le Cancer (UICC) (table 1). In five patients the carcinoma was 3–6 cm in greatest diameter, and in seven patients it was > 6 cm. One patient had multifocal carcinoma in the rectal, anal, and perianal areas. Clinical assessment of these patients prior to treatment included proctoscopy and sigmoidoscopy, estimation of hemoglobin, wbc count, platelet count, bilirubin, SGOT, alkaline phosphatase, and creati-

[1]Received Apr 28, 1981; revised Sept 8, 1981; accepted Sept 21, 1981.

[2]Departments of Radiation Oncology (*B. J. Cummings, W. D. Rider, A. R. Harwood, T. J. Keane,* and *G. M. Thomas*) and Medicine (*C. Erlichman* and *S. Fine*), The Princess Margaret Hospital, Toronto, Ontario, Canada.

[3]Secretarial assistance was supported by a grant from the Ontario Cancer Treatment and Research Foundation.

**Reprint requests to:* Dr. B. J. Cummings, Department of Radiation Oncology, The Princess Margaret Hospital, 500 Sherbourne St, Toronto, Ontario M4X 1K9, Canada.

TABLE 1.—UICC classification of squamous cell carcinoma of the anal canal (5)

T class	Criteria	No. of patients
Tis	Preinvasive carcinoma	0
T_0	No evidence of primary tumor	0
T_1	Tumor occupying no > ⅓ of the circumference or length of the canal and not infiltrating the external sphincter muscle	0
T_2	Tumor occupying > ⅓ of the circumference or length of the anal canal *or* tumor infiltrating the external sphincter muscle	1
T_3	Tumor with extension to rectum or skin but not to other neighboring structures	9
T_4	Tumor with extension to neighboring structures	3
T_x	Minimal requirements (clinical examination, radiography, and endoscopy) to assess the primary tumor cannot be met	0

nine and/or urea. Normal bipedal lymphangiograms were obtained in 11 of the 13 patients. Pelvic lymph node scintigraphy (6) was performed in seven patients, but patients were not excluded on the basis of the results of these studies.

The radiation therapy and chemotherapy were begun on the same day. Chemotherapy consisted of a single course of mitomycin (10 mg/m²) by iv bolus injection and 5-FU (1000 mg/m²/24 hours; maximum, 1500) by continuous iv infusion over 96 hours. An 82-year-old man received a 48-hour infusion only to a total of 1900 mg of 5-FU, and 10 mg of mitomycin; another patient received 15 mg of mitomycin rather than the calculated 17-mg dose. Otherwise, all patients received full doses of chemotherapy according to the schedule noted.

Radiation therapy was delivered by megavoltage equipment (25-mev photon radiation only in ten patients and 25-mev photons and cobalt-60 in three) to the primary tumor and to inguinal and pelvic lymph nodes. Because of the risk of involvement of pelvic lymph node groups situated relatively anteriorly in the obturator region (7) and the wish to irradiate the inguinal and external iliac node groups, treatment was begun by an anterior-posterior opposed pair of fields. After a midline dose of 3000 rads in 12 daily fractions, most patients then received further treatment by a lateral opposed pair of fields to the anal canal primary and low pararectal node groups only to a further 2000 rads in eight fractions. During this evolving study of combined radical radiation therapy and chemotherapy, no single treatment volume or field arrangement was used, but adjustments were made on the basis of previously noted toxicity. The first patient was treated by anterior and posterior radiation fields throughout the treatment course. Nine patients were treated by anterior and posterior fields initially, followed by laterally oriented fields as described above; three patients were treated by four-field techniques throughout. The upper border of the radiation fields was extended to approximately the level of the lower border of the first sacral segment in the first six patients until the dose of 3000 rads was reached. The fields were then reduced to the level of the bifurcation of the iliac artery at the lower border of the sacroiliac joint because of concern for possible hematologic toxicity. The seven patients treated subsequently had the upper border of the field set at the lower border of the sacroiliac joint throughout treatment. The lower border of the field was placed at least 3 cm below the anus. Most patients were treated in the supine position to diminish the effects of natural tissue contours around the buttocks and perineum. Wax bolus was applied over perianal tumor extension whenever appropriate. All fields were treated each day, and treatment was given without planned interruptions over 4 weeks in all patients except one. The 82-year-old male patient received planned split-course treatment over 6 weeks, and chemotherapy was given with the first phase of radiation therapy only.

Hemoglobin, wbc counts, and platelet counts were monitored weekly or more frequently during treatment, and until all acute gastrointestinal and skin reactions had subsided.

RESULTS

Toxicity

The major severe toxic effects from this treatment program were hematologic, gastrointestinal, and perianal skin reactions.

Significant acute hematologic toxicity occurred in four patients. In two patients the total peripheral wbc count decreased to < 2000/mm³ and in three patients the platelet count decreased to < 80,000/mm³ (one patient had both leukopenia and thrombocytopenia). In two patients the hemoglobin decreased by 4 g/100 ml at 4 and 5 weeks from the start of treatment, respectively, although bleeding or hemolysis could not be demonstrated (one of those patients had thrombocytopenia), and both received blood transfusion. All patients showed recovery of peripheral wbc and platelet counts to normal levels within 7 weeks of starting treatment. There was no correlation between the sizes of the radiation fields used and the hematologic toxicity observed.

Acute gastrointestinal toxicity developed during treatment in two patients in whom ileus and peritonism required interruption or cessation of radiation therapy; it was sufficiently severe to be considered life-threatening. In one patient this toxicity developed at 16 days (after 3250 rads) and in the other at 23 days (after 4500 rads) from the start of treatment. One other patient had severe diarrhea and fluid loss approximately 14 days after completion of treatment. All three patients recovered after appropriate supportive therapy. There appeared to be no correlation between radiation field sizes and gastrointestinal toxicity, but no small bowel radiologic series were obtained prior to irradiation in any of the patients.

It was noted that those patients who had severe gastrointestinal toxic effects were also those who had significant thrombocytopenia or leukopenia.

All 13 patients had moist desquamating perineal reactions at least 8 cm in diameter. Proctitis usually accompanied the perineal reaction although patients complained more of the perineal discomfort. Proctitis was managed by morphine derivatives and stool softeners. The perineal reactions were treated with analgesics, bland ointments or corticosteroid ointment, regular sitz baths, and bed rest. Because of the difficulty most patients experienced in managing the relatively inaccessible perineal reactions, inpatient care was arranged in all cases. Those patients admitted only for management of the perineal reaction and proctitis, compared to the more serious toxic effects noted above, remained in the hospital for an average of 22 days (range, 14–38).

Despite the various acute toxic reactions observed, treatment was completed without interruption in ten patients. Only one patient failed to complete the treatment planned, although she did receive a dose of 4500 rads to the tumor.

All patients followed $\geq$ 12 months had perineal skin changes, minor atrophy with telangiectasia, depigmentation, and, in some instances, subdermal fibrosis and irritation. Only one patient developed ulceration, and this healed after biopsy established that the ulcer was inflammatory rather than malignant. Three patients have had sufficient perianal discomfort to require regular use of skin emollients.

The three premenopausal patients experienced treatment-induced menopause.

Tumor Response

Complete tumor regression with preservation of anal continence was achieved in all patients. The rate of tumor regression was frequently rapid and almost complete by the end of treatment. Some residual thickening or ulceration persisted for up to 3 months in three patients.

Occasionally, some fibrous scarring remained within the anal canal but did not interfere with anal sphincter function. In the perianal region tumor regression left a superficial punched-out area with intact overlying epithelium.

In one patient an area at the anal verge broke down 8 months after treatment but biopsy showed chronic inflammation only. This patient also had poor perineal hygiene and this was thought to have contributed to the ulceration. No other patient had local ulceration or any other sign of possible tumor recurrence.

Thus, in all 13 patients, tumor control within the radiation fields has been maintained. Similarly, no patient has developed metastasis during the followup period.

DISCUSSION

This experience, although still limited both in patient numbers and in followup, appears to confirm the excellent response of squamous cell carcinoma of the anal canal to radiation therapy, 5-FU, and mitomycin. The relative rarity of this tumor prevents any single institution from carrying out an appropriate clinical trial comparing this therapy to either surgery or radiation therapy alone.

Earlier reports suggested that after mitomycin and 5-FU and preoperative radiation therapy to 3000 rads in 3 weeks, tumors < 4 cm in size might sometimes be treated by local excision rather than by abdominoperineal resection (8). The results reported here suggest that even larger tumors may be controlled by radical radiation doses and chemotherapy without surgery, since seven patients had carcinomas > 6 cm in greatest diameter.

Occasional responses to mitomycin and 5-FU without radiation therapy have been reported in the treatment of locally advanced or metastatic anal carcinoma (2,9). However, neither agent has a high level of activity when used alone against squamous cell carcinoma of the anal canal (10).

Previous experience in this institution with anal carcinomas of an extent similar to that of the carcinomas described in this report and treated by external radiation using techniques and doses similar to those described in this report resulted in a primary tumor control rate of 71% (ten of 14 patients) after radiation therapy alone. The crude 3-year survival rate was 70% for these patients treated by radiation therapy, and later surgery when necessary. Papillon (11) reported a 3-year crude survival rate of 76% in patients with stage T_3 and T_4 (UICC)

anal carcinomas following external and interstitial radiation therapy, and also employed subsequent surgery when necessary. These control rates following primary radiation therapy without chemotherapy indicate that it may prove difficult to demonstrate any superiority for the combined technique. Although all patients in this small series of 13 patients have achieved complete local tumor response with combined radiation therapy and chemotherapy, followup is still relatively brief, and recurrences may simply have been delayed by the combined treatment regimen.

The radiation fields used in this study were designed to include the inguinal, external, and internal iliac and pararectal nodes for at least part of the treatment course. The relative inaccessibility of many of these node groups to currently available methods of noninvasive examination causes difficulty in both staging and radiation treatment planning. The UICC guidelines (5) define the regional nodes of the anal canal as "the perirectal nodes and nodes distal to the origin of the inferior mesenteric artery" and recommend radiography for their assessment. However, conventional bipedal lymphangiography fails to demonstrate either the pararectal or the internal iliac node systems, although both are at risk. These lymph node systems are accessible to pelvic lymph node scintigraphy (6), and this procedure was performed in seven patients in this series (five studies were considered normal or equivocal and two were considered abnormal). This investigative technique is still undergoing evaluation, and surgical and pathologic correlations are not available for these patients.

The acute toxic effects observed with this combined radiation therapy and chemotherapy protocol might be decreased in several ways. It is not known whether the combination of radiation therapy with 5-FU and mitomycin is synergistic or simply additive. If the latter is the case, then sequential rather than simultaneous chemotherapy and radiation therapy might produce satisfactory tumor control rates but lower toxicity. Reducing the radiation dose rate from the 250 rads/day used in this series and prolonging overall treatment time to > 4 weeks might also reduce toxicity. Greater reductions in radiation field size for the latter part of the treatment program and the use of small bowel radiologic series during treatment planning to facilitate the shielding of small bowel within the pelvis are other possible ways of reducing the risk of severe gastrointestinal toxicity.

The degree of leukopenia and thrombocytopenia seen with this chemotherapy and radical radiation therapy has been greater than that reported after lower-dose preoperative radiation therapy (8). Also, severe acute gastrointestinal toxicity has not been reported when lower-dose radiation therapy was given preoperatively (2,3,8). It is not known whether the development of severe hematologic and gastrointestinal toxicity in the same patients occurred by chance. If this association of toxic effects is in any way causally related, leukopenia and/or thrombocytopenia might predict for acute gastrointestinal toxicity in these patients. It might then be appropriate to interrupt radiation treatment if either platelet or wbc count decreased significantly. Such interruptions may not necessarily reduce the likelihood of tumor control (11). However, a larger series of patients is needed to further study this possible association of hematologic and gastrointestinal toxicity.

The need for early abdominoperineal resection for the management of squamous cell carcinoma of the anal canal should now be questioned. Consideration should be given to deferring such surgery until after a trial of treatment with radical radiation therapy, 5-FU, and mitomycin which might preserve normal anal anatomy and function.

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High-Dose Doxorubicin: An Exploration of the Dose-Response Curve in Human Neoplasia [1,2]

Richard H. Wheeler,[3,*] William D. Ensminger,[3] James H. Thrall,[3] and Jeffery L. Anderson[3,4]

SUMMARY

Twenty-seven patients with advanced solid tumors (ten with lung cancer, eight with sarcoma, five with melanoma, four with miscellaneous tumors) were treated with high-dose doxorubicin (120 mg/m²) every 3 weeks for a total of 75 courses. As expected, marked myelosuppression was observed, with all patients having a granulocyte count nadir of < 500 cells/mm³. The median platelet count nadir was 91 × 10³/mm³ following the first cycle and 50 × 10³/mm³ following the fourth cycle. Moderate or severe stomatitis was seen with 65% of the courses, and 46% of the courses were complicated by fever > 101°F. Congestive heart failure was observed in only one patient after five cycles (600 mg/m²) of high-dose therapy. This patient had received 540 mg/m² of doxorubicin 2 years previously. Radionuclide ventriculography (MUGA) performed serially in 12 patients (eight of whom received 480 mg/m²) and clinical evaluation did not suggest an increased risk of cardiotoxicity at this dose rate. Overall, 11 of 24 (46%) evaluable patients responded (58% if patients with malignant melanoma are excluded). Responses included one complete and three partial responses in eight evaluable patients with lung cancer, one complete and two partial responses in seven evaluable patients with sarcoma, and no objective responses in five patients with malignant melanoma. With appropriate supportive care, repetitive courses of doxorubicin at a dose of 120 mg/m² can be given to patients of good performance status without a major increase in cardiotoxicity. However, the low complete remission rate, considering the observed toxicity, does not justify routine use of this regimen in patients with solid tumors.

[Cancer Treat Rep 66:493–498, 1982]

It is clear from studies performed with animal tumor models that most antineoplastic agents have a steep dose-response curve (1). A twofold increase in dose may produce up to a log increase in cell kill. Clinical studies have demonstrated the efficacy of high-dose therapy in sensitive human tumors such as the lymphomas and small cell lung cancer (1). A Southwest Oncology Group study comparing doxorubicin doses up to 75 mg/m² suggested improved response rates in patients with sarcomas, bladder cancer, and prostatic cancer who received the highest dose rate. Most of the complete remissions observed in this study were achieved with a dose of 75 mg/m² (2).

To further explore the dose-response curve for doxorubicin in human cancer, we initiated a trial of high-dose therapy. The dose chosen, 120 mg/m² every 3 weeks, was twice the commonly used dose of 60 mg/m². Three tumors were chosen for study: soft tissue sarcoma, for which doxorubicin is the most

[1]Received May 27, 1981; revised Oct 6, 1981; accepted Oct 20, 1981.

[2]Supported in part by Public Health Service grant 5-M0-1-RR-42 from the Division of Research Resources, National Institutes of Health, Department of Health and Human Services.

[3]Division of Hematology/Oncology and the Upjohn Center for Clinical Pharmacology (*R. H. Wheeler* and *W. D. Ensminger*), Division of Nuclear Medicine (*J. H. Thrall*), and Division of Cardiology (*J. L. Anderson*), Department of Internal Medicine, University of Michigan, Ann Arbor.

[4]*Present address:* LDS Hospital, Cardiology Division, Salt Lake City, UT.

**Reprint requests to:* Richard H. Wheeler, MD, Division of Hematology/Oncology, Department of Internal Medicine, S6360 University Hospital, Ann Arbor, MI 48109.

TABLE 1.—Patient characteristics, toxicity, and response*

Age (yrs)	Sex	Primary tumor	Prior therapy	No. of high-dose ADM courses	No. of courses with— Stomatitis > grade 2	No. of courses with— Fever > 101°F	Response
65	M	Lung	Surgery	3	1	1	SD
47	F	Lung	RT	4	2	0	PR
47	M	Lung	None	4	3	1	PR
40	M	Lung	RT	1	1	1†	NE
47	F	Lung	RT	2	2	0	SD
41	F	Lung	None	1	1	1	PD
52	F	Lung	RT, methyl-CCNU	3	2	2	PR
37	M	Lung	RT, CTX	2	0	0	SD
50	M	Lung	None	1	0	1†	NE
56	F	Lung	None	3	3	3	CR
52	F	STS	Surgery	3	2	2	SD
57	M	STS	None	6	2	1	SD
48	M	STS	Surgery	2	1	0	SD
21	M	STS	Surgery, ADM	5	2	1	PR
46	M	STS	Surgery	4	4	1	CR
24	F	STS	Surgery	4	4	4	NE
26	M	Chondrosarcoma	None	4	4	4	PR
61	M	Osteosarcoma	None	2	1	2	PD
31	F	Melanoma	Surgery	4	2	2	SD
49	M	Melanoma	Surgery, ActD + DAC	2	0	0	SD
56	M	Melanoma	Surgery	1	0	0	PD
58	M	Melanoma	Surgery, DAC	1	1	1	PD
54	F	Melanoma	Surgery	2	2	0	SD
56	F	Ovary	ADM + cisplatin	3	3	3	CR
54	M	Lacrimal gland	Surgery, RT, MTX/CP	4	4	0	CR
55	M	Bladder	RT	3	1	2†	PR
55	M	Prostate	DCMTX	1	1	1†	PR
			Total	75	49	34	

*STS = soft tissue sarcoma; RT = radiation therapy; CTX = cyclophosphamide; ADM = doxorubicin; ActD = dactinomycin; DAC = dacarbazine; BHD = carmustine (BCNU), hydroxyurea, dacarbazine; MTX/CP = methotrexate/carboxypeptidase; DCMTX = dichloromethotrexate; SD = stable disease; PD = progressive disease; NE = not evaluable.

†Died during therapy.

effective single agent; melanoma, as representative of a resistant tumor; and non-small cell lung cancer, which has a modest response rate (3). This spectrum of tumor sensitivity to doxorubicin was chosen on the premise that an increased efficacy of high-dose therapy could be demonstrated by production of a high rate of complete remissions in sensitive tumors or by attainment of objective responses in tumors routinely resistant to standard doses.

PATIENTS AND METHODS

The 27 patients with refractory solid tumor malignancies included in this study are listed in table 1. There were ten females and 17 males, with a median age of 50 years and a median Karnofsky performance status of 80%. Seven patients had received prior radiation therapy, and eight had received prior chemotherapy; 15 received high-dose doxorubicin as their first nonsurgical therapy.

Pretreatment requirements for entry in this protocol included a Karnofsky performance status of at least 70%, serum bilirubin < 1.5 mg/100 ml, serum creatinine < 1.5 mg/100 ml, wbc count ≥ 4000/mm^3, platelet count ≥ 150,000/mm^3, and objectively measurable disease. Written informed consent was obtained from all participants. This study was approved by the University of Michigan Committee to Review Clinical Investigation Involving Human Beings.

Cardiac function was assessed by radionuclide ventriculography, as previously described from our institution (4), prior to administration of the first dose of doxorubicin and after cumulative doses of 240 and 480 mg/m^2.

A complete remission (CR) was defined as the clinical disappearance of all detectable disease. A partial remission (PR) was defined as a 50% decrease in the sum of the products of perpendicular diameters of all measurable lesions for at least 1 month, and no evidence of new lesions. Stable disease was defined as a < 50% decrease or a < 25% increase in the sum of the products of perpendicular diameters. Progressive disease was defined as a 25%

increase in the sum of the products of perpendicular diameters of all measurable lesions for at least 1 month, and no evidence of new lesions. Student's t test was used for comparison of mean values.

RESULTS

The objective responses to high-dose doxorubicin are shown in table 1. Twenty-four patients were evaluable for response. Two patients with lung cancer died during the first course of therapy before tumor response could be assessed. One patient with soft tissue sarcoma had postoperative residual disease in the mediastinum but no measurable lesion. There were four CRs and seven PRs, for an overall response rate of 46%. One patient with lung cancer had a CR of the primary tumor and of hepatic metastases lasting 8 months. She subsequently died from CNS involvement by the tumor. Although initially called poorly differentiated adenocarcinoma, the diagnosis was changed to small cell undifferentiated at the final pathology review.

Two of five evaluable patients with soft tissue sarcoma achieved a response. The CR has lasted 10+ months with no additional therapy following the four courses of doxorubicin. The partial responder had received adjuvant doxorubicin 2 years previously at the time of primary surgical management.

CRs were noted in one patient with ovarian carcinoma and in one patient with extensive loco-regional spread (including brain invasion) of a lacrimal gland carcinoma. The patient with ovarian cancer had failed to respond to two courses of doxorubicin (60 mg/m^2) and cisplatin (80 mg/m^2) prior to receiving high-dose doxorubicin. The CR attained with high-dose doxorubicin lasted 8 months, with no therapy following three high-dose courses.

As anticipated, the myelosuppression of this regimen was severe. All patients had granulocyte count nadirs < 500/mm^3. The granulocyte count usually decreased to < 1000/mm^3 7–9 days after administration of doxorubicin, and routinely recovered between Days 16 and 20. Myelosuppression was cumulative with subsequent courses as demonstrated by a progressive decrease in the platelet count nadir and the duration of granulocytopenia. The duration of granulocytopenia (< 1000 cells/mm^3) increased from a mean of 6.8 days following course 1 to 10 days following course 4 ($P < 0.01$). Marked thrombocytopenia was also observed, with a median platelet count nadir of 90,000/mm^3 following the first course and 53,000/mm^3 following the fourth course ($P < 0.05$). No overt bleeding episodes were observed. Patients who received three or more courses often required transfusion to maintain the hematocrit at 30%.

Febrile episodes (oral temperature > 101°F) occurred during 46% of the courses and were presumed to have an infectious etiology. Three patients had positive blood cultures, and all three died of infectious complications. A fourth patient insisted on hospitalization at his community hospital. Antibiotics were not administered and the patient died 48 hours later. The three patients who died of infectious complications despite antibiotic treatment all had a performance status of < 70% and were treated late in this trial. Two other patients with low performance status (40%–60%) were included in this series; neither developed fatal infections but had progressive disease after one cycle of therapy.

Nausea and vomiting were mild to moderate and were generally controlled with antiemetics. No patient refused further therapy because of this side effect, and most drug administrations were on an outpatient basis. Moderate or severe stomatitis complicated 65% of the courses. The severity generally increased with subsequent courses. Although oral intake was somewhat impaired for 3–5 days, no patients required admission for hydration or alimentation.

A major concern at the inception of this study was the possibility of an increased incidence of cardiotoxicity. One patient who 2 years previously had received 540 mg/m^2 of doxorubicin as adjuvant therapy received high-dose doxorubicin for recurrent disease. Four weeks following his fifth dose of high-dose doxorubicin (600 mg/m^2), he developed signs of cardiac failure. Shortly thereafter, he died of progressive neoplastic disease. Autopsy revealed extensive myocardial damage. The cumulative doxorubicin dose was 1140 mg/m^2. None of the other patients developed clinical signs or symptoms suggestive of cardiac toxicity. Nine patients received 480 mg/m^2 of high-dose doxorubicin. Two of these patients received an additional 240 or 180 mg/m^2, respectively, at dose rates of 60 or 90 mg/m^2/course, and a third patient received two additional courses of high-dose therapy.

Radionuclide ventriculography was performed serially in 12 patients, including eight who received at least 480 mg/m^2 (fig 1). One patient was evaluated only after receiving 480 mg/m^2; the ejection fraction (EF) was 71%. The mean resting EF for all patients was 65% before therapy, 62% after receiving 240 mg/m^2, and 57% after receiving 480 mg/m^2 ($P > 0.05$). For the eight patients who received four cycles of therapy, three had no change after receiving 480 mg/m^2 and five had a decline in the EF. One of the patients who had no change was the patient who developed congestive heart failure. Two patients

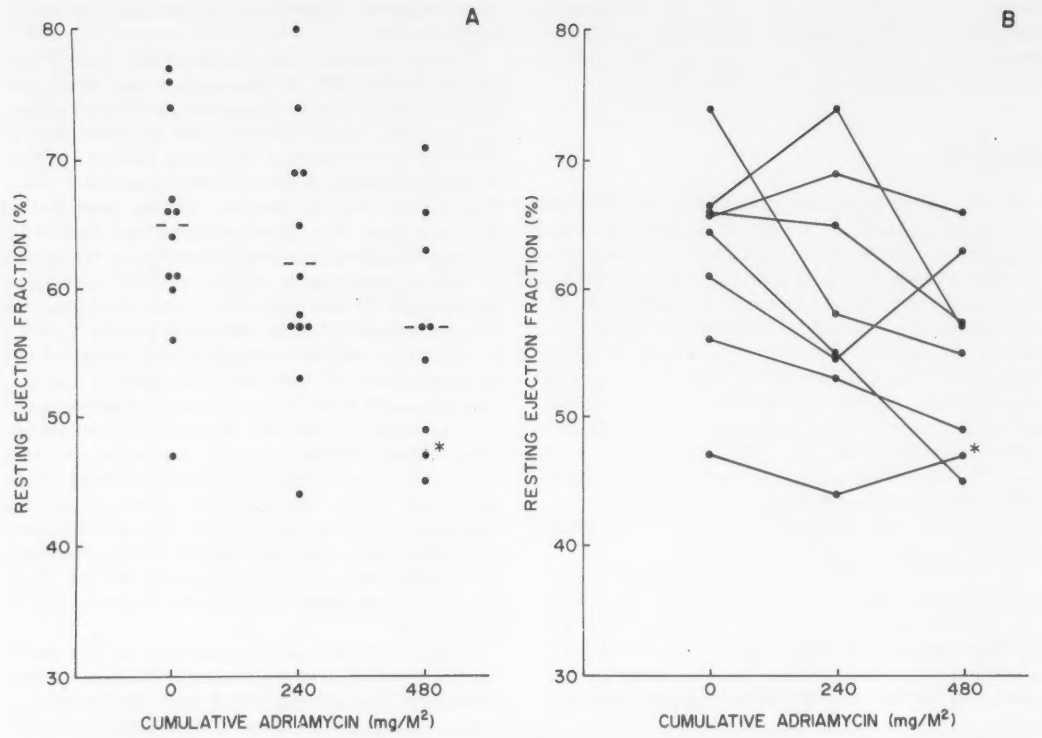

FIGURE 1.—Cardiotoxicity of high-dose doxorubicin as shown by radionuclide ventriculography. A) Resting EF determinations for 12 patients. B) Sequential resting EF determinations in patients receiving 480 mg/m². * indicates patient developed cardiac failure.

who had EF reductions from a value of 56% to 49% and from 74% to 55% underwent endomyocardial biopsy. Using the Stanford grading system (5), the biopsies were both graded 1+ (scanty cells showing early myofibrillar dropout or swelling of the sarcoplasmic reticulum or both) in electron microscope preparations. A third patient had a decrease in EF from 67% to 57%. An endomyocardial biopsy was graded as 3+ (diffuse myocyte damage). This patient received two additional doses of doxorubicin at a dose of 90 mg/m² without evidence of congestive heart failure or further reduction in EF. The greatest decrease was seen in a 57-year-old male whose EF went from 64% to 45% after four courses. He subsequently received an additional 240 mg/m² of high-dose therapy without evidence of cardiac failure or further reduction in EF.

DISCUSSION

Doxorubicin is an antitumor antibiotic with a wide spectrum of activity in human cancer (3). Like many antineoplastic agents, doxorubicin has a steep dose-response curve in animal tumor and in vitro systems (6–8). The major mechanism of action appears to be intercalation between adjacent base pairs of the DNA double helix, with inhibition of DNA and RNA polymerase-mediated reactions (9). Additional studies have suggested both a concentration dependence of the biochemical lesion produced and the possibility of additional mechanisms at higher drug concentrations (10–13).

In addition to the laboratory data suggesting the potential efficacy of high-dose doxorubicin therapy, clinical studies have also suggested a dose-response effect. O'Bryan et al (2) compared doses of 45–75 mg/m² in good-risk patients; a tendency to better remission rates with the high-dose schedule was noted in patients with sarcomas and bladder and prostate cancers. Also, 64% of the CRs occurred with the high-dose schedule. Cortes et al (14) observed that decreasing the dose of doxorubicin during adjuvant treatment of osteogenic sarcoma is associated with a greater risk of recurrence. Nagel

(15) has shown that single doses in the range of 120–240 mg/m² can be safely administered with appropriate supportive care. There were five CRs and six PRs in the 14 patients studied. Seven of the responding patients had failed to respond to conventional doses of doxorubicin. Toxic effects noted in these 14 patients included a nadir wbc count < 1000/mm³ in ten patients and stomatitis in 12. One patient developed acute heart failure 24 hours after the doxorubicin injection, which resolved after 4 days. The long-term consequences of repetitive high-dose therapy have not been previously determined.

The overall response rate of 46% with 17% CRs, which we observed in this study, does not confirm Nagel's reported experience. Excluding the patients with melanoma from this series, the overall response rate was 58% with 21% CRs. The 43% response rate (three of seven patients) in non-small cell lung cancer and 38% response rate (three of eight patients) in sarcoma have been approached with doxorubicin given in more conventional doses (3). The lack of antitumor effect in patients with malignant melanoma suggests that a twofold increase in dose rate will not be effective in resistant tumors.

This study does demonstrate that repetitive courses of high-dose therapy can be given to patients with good performance status. Three of the four CRs that occurred were in patients with tumors considered responsive to doxorubicin. Nagel (15) observed seven responses in patients resistant to conventional doses of doxorubicin (two with lymphoma, two with sarcoma, and one each with breast, lung, or parotid cancer). Our patient with ovarian cancer attained a CR after failing to respond to conventional doses of doxorubicin. We have also observed responses in patients with lymphoma resistant to doxorubicin-containing regimens (16). Yagoda et al (17) reported a response to 75 mg/m² in a patient with bladder cancer who progressed while receiving 45 mg/m². These observations suggest that there is a patient population that could be effectively treated with this high-dose regimen. A higher rate of CR may be attainable with high-dose therapy in traditionally responsive tumors. A 100% CR rate has been observed in evaluable patients with non-Hodgkin's lymphoma treated with high-dose doxorubicin, vincristine, and prednisone (18). The tumor cloning assay may also permit identification of these patients. Ozols et al (19) demonstrated an antitumor effect of doxorubicin administered ip in two patients who had in vitro resistance to low concentrations of doxorubicin but sensitivity to higher concentrations.

Doxorubicin dose rate may also have an important effect on the development of cardiotoxicity. Weiss et al (20) found a lower than expected incidence of cardiomyopathy with weekly therapy. Similar findings have been reported by others (21,22). The possibility thus existed that the high-dose bolus program could be associated with an increased risk of cardiotoxicity. We observed one instance of clinical cardiac failure after 600 mg/m² of high-dose doxorubicin in a patient who had received 540 mg/m² 2 years previously. No other patient had clinical evidence of failure, including one patient who received 660 mg/m² (480 mg/m² in high doses) and two who received 720 mg/m² (480 and 720 mg/m² in high doses, respectively). The duration of followup after the last dose of doxorubicin was at least 6 months in these patients. We did not observe the acute, reversible cardiac failure described by Nagel (15).

Alexander et al (23) used first-pass radionuclide angiocardiograms to study 55 patients receiving doxorubicin. A significant decline in EF from 62% to 50% was found in eight patients who received 450–549 mg/m². The change in mean EF in the eight patients in our study who received 480 mg/m² was from 65% to 57% ($P > 0.05$), suggesting that the high-dose program does not produce increased cardiotoxicity. Ritchie et al (24) also found no correlation between resting EF and cumulative doxorubicin dose.

Predictive criteria for doxorubicin cardiotoxicity have been proposed by Alexander et al (23). Using these criteria, three patients had no cardiotoxic effects (< 10% decrease in EF). After receiving 480 mg/m², one of the three patients developed cardiotoxic effects with the next course of high-dose doxorubicin; another patient has received an additional 240 mg/m² at a lower dose rate without cardiotoxic effects. Four patients had mild cardiotoxic effects (> 10% decline in EF, with the last EF > 45%). Three of these patients received an additional 180 mg/m² of doxorubicin without further evidence of toxicity. One patient had moderate toxic effects (> 15% decrease in EF, with the last EF < 45%). This patient refused endomyocardial biopsy. He received an additional 240 mg/m² of high-dose doxorubicin without developing cardiac abnormality. Although this group of patients is select and small, these findings suggest that the use of the resting MUGA and possibly the endomyocardial biopsy to limit the cumulative dose of doxorubicin may exclude from treatment patients who are capable of receiving further therapy.

The major purpose of this study was to determine the efficacy and toxicity of intensive high-dose therapy with doxorubicin. We have demonstrated that with appropriate supportive care, repetitive courses at a dose rate of 120 mg/m² by iv bolus every 3 weeks can be administered to patients of good per-

formance status without an apparent increased risk of cardiotoxicity. The overall toxicity without a dramatic improvement in the CR rate does not support the use of this program as routine therapy in patients with non-small cell lung cancer, soft tissue sarcoma, or melanoma. We do plan further trials of this dose rate in patients with tumors highly responsive to standard doses of doxorubicin.

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Comparative Effects of Methotrexate, Two Nonclassic Folic Acid Antagonists, and Cytarabine on Hematopoietic Precursor Cells[1,2]

John C. Marsh[3,4,*]

SUMMARY

The effects of three antifolates, methotrexate (MTX), triazinate (TZT), and a trimethoxyquinazoline derivative (TMQ), and of cytarabine (ara-C) on leukocyte-committed colony-forming cells from three species were compared using an assay allowing in vivo metabolism in an agar diffusion chamber. Dose-survival curves approximated plateaus, and the level of the plateau was variable among species. Canine marrow colony-forming cells were less sensitive to MTX (about 90% survival) than those from mice or man (25%–40% survival). Human marrow colony-forming cells were less sensitive (70% survival) to TZT than those from mice or dogs (25% survival). No interspecies differences were found for TMQ or ara-C. Mouse marrow in situ was less sensitive to iv MTX than mouse marrow in the chamber. No such difference was found for iv TMQ or ara-C. This comparison could not be made for ip TZT.

[Cancer Treat Rep 66:499–504, 1982]

The classic dihydrofolate reductase (DHFR) inhibitor methotrexate (MTX) has been used as an anticancer agent for approximately 30 years. Efforts to devise antifolates with greater therapeutic indices have resulted in a variety of compounds with significantly greater activity than MTX against some experimental tumors. These include the nonclassic agents triazinate (TZT) (1) and the 2,4-diamino-6-[(anilino)methyl] quinazoline derivative TMQ (2). Both compounds are slightly less active than MTX against purified L1210 DHFR. TZT is more active against the rat Walker 256 carcinosarcoma, while TMQ is more active against L1210, L5178Y, Sarcoma 180, and Walker 256 in vitro and against B16 melanoma and Colon 26 and Colon 38 tumors in vivo. The increased activity of both agents against these tumors may be due, at least in part, to increased efficiency of intracellular transport relative to MTX (1,2). Unlike MTX, TMQ blocks the incorporation of uridine and thymidine into nucleic acids of P388 cells and is a more effective inhibitor of deoxyuridine incorporation (3). TZT has been evaluated in phase I (4,5) and II (6) clinical trials, while TMQ is still in the preclinical developmental stage.

Because one of the major toxic effects of the antifolates is myelosuppression, it was decided to compare the effects of MTX, TZT, and TMQ on leukocyte-committed bone marrow colony-forming cells from mouse, dog, and man, using the agar diffusion chamber precursor cell (ADCPC) colony-forming assay (7,8). This allows in vivo metabolism of the drugs under study. Cytarabine (ara-C) was used to

[1]Received July 1, 1980; revised Aug 24, 1981; accepted Sept 18, 1981.

[2]Supported by grant CH-37 from the American Cancer Society and by Public Health Service grants CA-18341 and CA-08341 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Departments of Medicine and Pharmacology, Yale University School of Medicine, New Haven, CT.

[4]I am grateful to Marianne Nierenburg, Barbara Brown, and Alison Tracz for excellent technical assistance; to Dr. Joseph Bertino for the supply of TMQ, as well as a critical review of the manuscript; and to Dr. Diana Fischer, who provided helpful statistical discussion.

*_Reprint requests to:_ Dr. John C. Marsh, Department of Internal Medicine, Yale University School of Medicine, 333 Cedar St, New Haven, CT 06510.

evaluate the relative proliferative rates of ADCPCs from the different species.

Preliminary reports of these studies have been presented (9,10).

MATERIALS AND METHODS

Experimental animals, bone marrow preparation, and culture techniques were as previously described (11). Because of the relatively low cell-killing activity of the agents tested, chambers containing marrow exposed to the drug were *not* plated with an increased number of cells, as is necessary if the surviving fraction is reduced by several orders of magnitude.

Drugs

MTX (Lederle Laboratories, Pearl River, NY) was obtained in a concentration of 25 mg/ml. Further dilutions were in sterile isotonic saline. Ara-C (Upjohn Company, Kalamazoo, MI) (100 mg) was diluted with sterile saline. TZT was obtained from the National Cancer Institute (Bethesda, MD) in 250-mg vials, which were initially diluted with 10 ml of sterile water. Further dilutions were with sterile isotonic saline. TMQ was obtained from Dr. J. R. Bertino. It was initially dissolved in 0.1 N HCl at a concentration of 12 mg/ml and was further diluted to 4 mg/ml with sterile saline.

Experimental Design

For in situ experiments, varying doses of the antifolates were injected iv or ip into normal mice. Eighteen hours later, femoral marrow was removed from five mice at each dose level. The marrow was pooled, counted, and placed in agar diffusion chambers, which were implanted in the peritoneal cavities of normal mice. In one TZT experiment, a 3-hour interval was also used. In the ara-C experiments, the interval was only 1 hour because it has been reported that a comparison of relative marrow proliferative states was obtained with this time interval (12).

For experiments in which marrow was to be exposed in the diffusion chambers, mouse, dog, or human marrow in agar was placed in the chambers, which were then implanted in normal unirradiated mice. After 2–4 hours, the drugs were injected iv (TZT was injected ip) into five mice at each dose level. TZT was given ip because of the immediate lethality of iv doses > 10 mg/kg. Eighteen hours later (1 hour later in the ara-C experiments), the chambers containing mouse and dog marrow were transplanted to unirradiated mice and those containing human marrow were transplanted to irradiated mice for the appropriate duration. Colony development from human marrow does not occur unless the host mice are irradiated.

Incubation times following final chamber implantation were 7 days for mouse marrow, 9 days for canine marrow, and 14 days for human marrow since colony development is optimal at these times. At the end of this period, the chambers and the Millipore filters were removed, and the colonies were counted under a dissecting microscope. Both small (eight to 50 cells) and large (> 50 cells) colonies were counted. The data are derived from the total colony number.

For each drug dose, the mean and SE of the survival fraction of colony-forming cells were calculated.

RESULTS

MTX

The ADCPC survival in the chamber 18 hours after iv MTX was similar for mouse and human marrow (fig 1), with an apparent plateau at 25%–40% survival over the dose range studied. Dog marrow was somewhat less sensitive at the lower doses, but the survival approached that of the other species at 300 mg/kg (47% survival).

Mouse marrow was exposed in situ to MTX injected iv and was then assayed for surviving ADCPCs; there was essentially no effect, except for reduction of survival to 78% at a dose of 300 mg/kg (table 1). The effect of ip injected drug was somewhat greater but the difference was minimal. Femoral marrow cellularity was only slightly reduced when the drug was given by either route.

The effect of iv MTX on mouse ADCPC survival was greater in the chamber than in situ at each dose studied.

TMQ

At iv doses of 5–30 mg/kg, the survival of bone marrow ADCPCs from all three species exposed 18 hours in the chamber ranged from 68%–131%, with no apparent difference between species or doses (fig 2). Doses > 30 mg/kg iv in the chamber-bearing mice caused immediate death, preventing the study of higher doses with this route of administration.

Doses up to 100 mg/kg ip were well-tolerated and an 18-hour dose-response study for mouse marrow ADCPCs in situ showed an apparent plateau at 60% survival at doses of 30–100 mg/kg. There was no

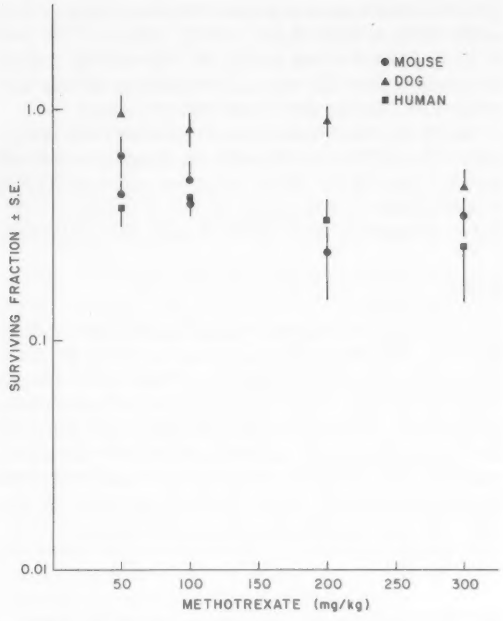

FIGURE 1.—Dose-survival curves of bone marrow ADCPCs from different species 18 hrs after MTX. Bone marrow was suspended in diffusion chambers in agar; drug was injected iv into the mice bearing the chambers, with transplantation to untreated mice 18 hrs later. Each symbol represents 1 experiment. Some error bars were omitted to avoid visual crowding.

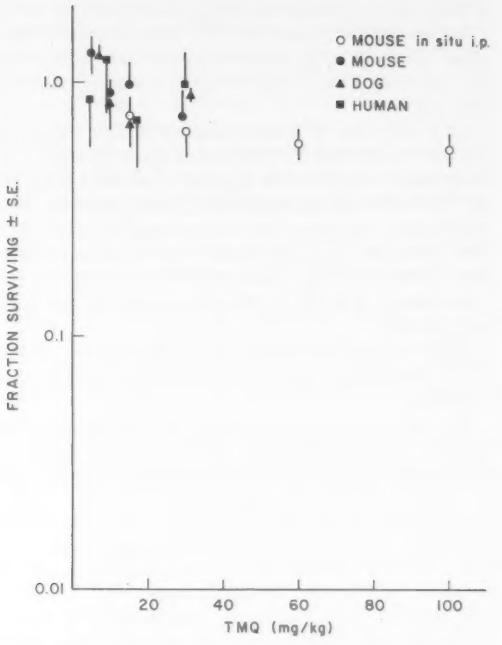

FIGURE 2.—Dose-survival curves of bone marrow from different species 18 hrs after iv TMQ. Dark symbols: experiments performed as described in fig 1 legend. Light symbols: drug administered ip to normal mice whose femoral marrow was assayed for surviving ADCPCs 18 hrs later. Some error bars were omitted to avoid visual crowding.

difference in mouse marrow ADCPC survival between those exposed in the chamber or in situ at 15 and 30 mg/kg.

Femoral cellularity was reduced very slightly to 95% by doses of 15 and 30 mg/kg ip and to 92% by doses of 60 and 100 mg/kg.

TZT

Doses > 10 mg/kg were immediately fatal when injected iv. Accordingly, all experiments were done with drug given ip, which was tolerated at doses of 30–90 mg/kg during the 18 hours of the experiment.

TABLE 1.—Effects of MTX on mouse ADCPC survival in situ and on marrow cellularity 18 hrs after administration

Dose (mg/kg)	Survival fractions*		Cellularity (% of control)	
	iv	ip	iv†	ip‡
50	1.18 ± 0.32	104.7 ± 0.21§	84.1	95.6 ± 5.0
100	0.95 ± 0.22	0.81 ± 0.14§	89.9	93.7 ± 3.3
200	0.99 ± 0.25	0.82 ± 0.09¶	105	93.9 ± 5.0
300	0.78 ± 0.18	0.60 ± 0.14¶	92.4	92.4 ± 3.2

*Based on concentration (surviving colonies/No. of cells plated); values = mean ± SE.
†Pooled cellularity of 1 femur from each of 5 mice/group.
‡Same as † but pooled data from 4 experiments; values = mean ± SE.
§Mean of 3 experiments.
¶Mean of 2 experiments.

Under these conditions, when bone marrow in the chamber was exposed to TZT, human marrow was least affected, with a survival plateau of 70%, while the survival plateau for mouse and dog marrow was 25% (fig 3).

The survival of mouse marrow ADCPCs in situ was little affected by TZT doses up to 75 mg/kg but decreased to 54% at the highest dose (90 mg/kg) (fig 3). The survival fractions for in situ marrow were all higher than those for mouse marrow exposed in the chamber, but this could well be explained by the relatively high concentration of drug bathing the chambers in the peritoneal cavity in the latter experiment.

Equivalent amounts of TZT and MTX administered ip were similar in their effects on in situ mouse marrow.

To take into account changes in total marrow cellularity, the data from the in situ experiments were expressed as ADCPCs surviving per femur; a progressive decrease was found. The survival fractions were 0.90 ± 0.08, 0.81 ± 0.07, 0.65 ± 0.09, and 0.35 ± 0.03 at doses of 30, 60, 75, and 90 mg/kg, respectively, while the corresponding cellularities were 71%, 103%, 59%, and 65% of control.

An in situ experiment over the same dose range, with a 3-hour interval between ip drug injection and assay, showed no effect on stem cell survival or femoral marrow cellularity (survival fraction, 0.88–1.14; cellularity, 89%–104% of control).

Ara-C

To evaluate the relative proliferative states of the marrow ADCPCs from the three species, the surviving fractions were measured 1 hour after administration of ara-C (100–300 mg/kg) (12). For marrow exposed to iv drug in the chamber, the survival curves approximated plateaus at 70%–90% for mouse marrow, 55%–70% for dog marrow, and 40%–60% for human marrow (fig 4). At the highest dose, human marrow appeared to be more sensitive.

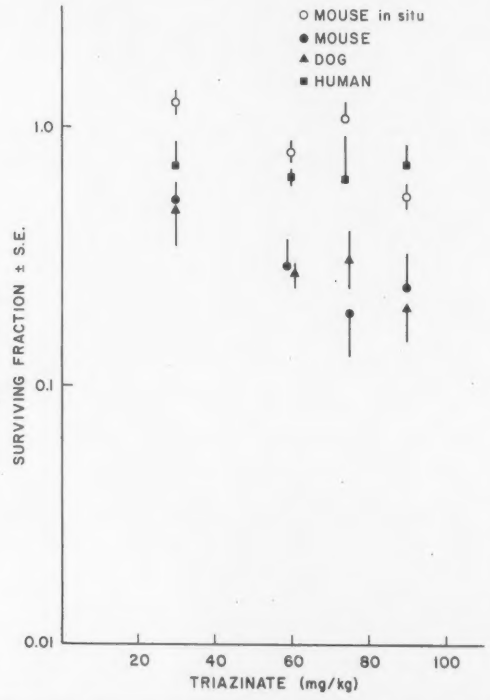

FIGURE 3.—Dose-survival curves of bone marrow from different species 18 hrs after ip TZT. Dark symbols: experiments performed as described in fig 1 legend, except drug was given ip. Light symbols: experiments performed as described in fig 2 legend. Some error bars were omitted to avoid visual crowding.

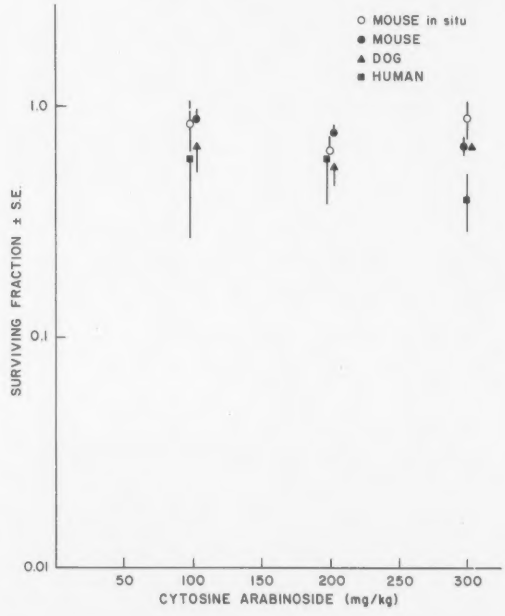

FIGURE 4.—Dose-survival curve of bone marrow from different species 1 hr after iv ara-C (cytosine arabinoside). Dark symbols: experiments performed as described in fig 1 legend, except transplantation was at 1 hr. Light symbols: drug administered iv to normal mice whose femoral marrow was assayed for surviving ADCPCs 1 hr later. Some error bars were omitted to avoid visual crowding.

For in situ mouse marrow 1 hour after iv administration of ara-C, stem cell survivals were 65%–89%, which resembled those for mouse marrow in the chamber. Cellularity was affected only at the highest dose (300 mg/kg), which reduced it to 71%. When the drug was given ip, there was no stem cell killing or change in femoral cellularity (data not shown).

DISCUSSION

This technique has been used to study the comparative sensitivity of granulocyte-macrophage precursor cells from different species to various anticancer drugs (8,11,13). The results of such experiments must be interpreted in view of the fact that the drugs are being given to and metabolized by mice, in which the pharmacokinetics may differ from those of dogs or humans. Previous studies (9,10) have shown that species sensitivities may differ unpredictably for various drugs. The sensitivity of marrow suspended in the chamber may also differ in some instances from that in situ; the comparative mouse experiments were done to provide perspective on this question for each agent.

Each of the three antifolate drugs studied, as well as ara-C, produced dose-survival curves that were plateaus rather than being exponential with dose, suggesting that the agents are phase-specific. The sensitive portion of the ADCPC population represents only a small fraction of the total population over the time the cells were in contact with the drug, presumably those cells that were in the S phase of the cell cycle. The 1-hour ara-C experiments suggested that the order of sensitivity to phase-specific drugs might be human > dog > mouse, although the differences were generally very small. This order of sensitivity did not hold for the 18-hour experiments with the antifolic drugs, suggesting that other factors, such as the longer time of exposure, pharmacokinetics, drug-induced delays in passage through the cell cycle, and difference in cellular uptake, may be more important determinants than differences in cell cycle time, length of the S phase, or growth fraction.

The results of studies by Gordon and Blackett (8) of mouse and human marrow ADCPC sensitivities to MTX differ somewhat from the results in this study. Whereas I observed a plateau at 25%–40% survival for human and mouse marrow in the chamber, they obtained an exponential dose-response curve for both species, with a fractional survival of < 10% at a dose of 300 mg/kg. When agar was omitted from the chambers during the drug exposure in their experiments, survival of ADCPCs from both species approached a plateau between 50% and 60%. The explanation for this difference is not obvious. Possible factors are differences in individual cell cycle proliferative kinetics in man, the use of different mouse strains, or the use of different agar and the degree of drug binding to it. The dose-survival curves for mouse marrow in situ exposure are quite similar in both studies, with a plateau at approximately 80% survival. No difference in sensitivity between human and mouse colony-forming cells was observed in either study.

Mouse marrow colony-forming cells in the chamber were more sensitive to MTX than those in situ. This was also seen in the studies of Gordon and Blackett (8). For several other drugs such as 5-FU (8,10), 5-azacitidine (10), and AMSA (J. C. Marsh, unpublished data), the reverse has been observed: marrow in situ is *more* sensitive than marrow in the chamber.

TMQ is a nonclassic antifolate (14) with potent inhibitory activity against DHFR from human leukemia cells and MTX-resistant L1210 mouse leukemia cells (2). MTX and TMQ given ip were comparable in their effects on mouse marrow ADCPCs in situ, producing a plateau at 60% survival. Low doses of TMQ were slightly less active than low doses of MTX against marrow colony-forming cells from mice and man in the chamber but the differences were small.

The effects of low-dose TMQ on ADCPC survival were similar in all three species; this agent has not yet been used clinically, but dogs (15) and mice (2) have tolerated up to 60 and 180 mg/m^2, respectively. It may be predicted that the hematologic toxic effects in man after single doses will be similar to those in the mouse or dog, if the pharmacokinetics are comparable.

TZT was more effective in killing mouse or dog ADCPCs than those from human marrow. It was the only agent tested by ip injection in chamber-bearing animals, and thus the marrow was exposed to higher concentrations of drug than if iv administration had been possible. Nevertheless, the differences in species sensitivity are clear; human marrow is the least sensitive. In contrast, canine marrow ADCPCs were least sensitive to MTX when tested in the same way.

The acute toxicity of TZT injected iv, compared to the ip route, may have been related to the pH of the resulting solution or to the capacity of the drug itself to cross the blood-brain barrier in high concentration and produce respiratory or cardiac arrest. Acute reactions consisting of ataxia, dyspnea, coma, and death have been observed in dogs and monkeys after iv injection of doses > 5 mg/kg (16). Neurologic toxic effects have been observed in humans (5).

Toxicologic studies of TZT indicate an LD10 for mice at doses between 192 and 384 mg/m² (LD100).[5] One of two dogs given 800 mg/m² died immediately; the other, killed on Day 7, had a hypocellular marrow.[6] A maximum tolerated human dose was in the range of 500 mg/m², with mild leukopenia in one of ten patients at that dose (5). This suggests that the order of marrow sensitivity to single doses is mouse > dog > human, although the data are incomplete. This is similar to the species sensitivity seen in the present study (mouse = dog > human).

No differences between species were found in the effect of ara-C on marrow colony-forming cells in the chamber 1 hour after an iv injection, suggesting that the proliferative states of these populations are similar in the different species (12,17).

The degree to which the dose-survival curve for mouse marrow ADCPCs in the chamber resembles that obtained for mouse marrow in situ, for a particular drug, increases the likelihood that the results for canine and human marrow can be extrapolated to predict the relative myelosuppression for these species in vivo. For TMQ and ara-C, the mouse data are not different for the two methods. For MTX, marrow in the chamber was more sensitive than that in situ after an iv dose; this difference, observed also by Gordon and Blackett (8), was decreased by omission of agar from the chamber in their studies, suggesting that the agar may bind the drug, thus increasing its bioavailability over the period of incubation in the secondary, non-drug-treated recipient. A similar difference between marrow in situ and that in the chamber was seen after ip TZT in this study, but this could be attributed to a different route of administration.

Although all four agents tested against marrow in the chamber produced survival curves with plateaus, the levels differed among the agents and species in several instances. Because the pharmacokinetics were identical for a given agent, species differences in cellular sensitivity could be observed. It is clear that inherent differences in committed colony-forming cell sensitivity to anticancer drugs exist between different species and that they vary for different drugs. This factor, in addition to differences in bone marrow reserve, will determine the clinical marrow tolerance and the ability to predict it from preclinical toxicologic studies.

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Effect of Vinblastine, Vincristine, and Vindesine on Neutrophil Phagocytosis and Iodination [1,2,3]

Veronica Chohan and Raymond M. Lowenthal [4,5,*]

SUMMARY

The ability of neutrophils to phagocytose *Saccharomyces cerevisiae* and iodinate *Staphylococcus aureus* and *Candida albicans* was evaluated when the neutrophils were incubated with therapeutic and above-therapeutic concentrations of vinblastine, vincristine, or vindesine. The phagocytic *capacity*, but not the phagocytic *rate*, was decreased in the presence of vincristine but not the other drugs. Above-therapeutic concentrations of vinblastine and vincristine reduced the iodination of *C. albicans* when compared with the control. We concluded that therapeutic doses of these drugs were not detrimental to important neutrophil functions.

[Cancer Treat Rep 66:505–508, 1982]

Patients with many types of malignant disease have an increased risk of infection. In patients undergoing cytotoxic chemotherapy, neutropenia is a major factor contributing to this increased risk (1), and the possibility that drug-induced defects of neutrophil function may also be contributing factors must be considered. Several drugs have been shown to alter in vitro neutrophil functions such as phagocytosis (2,3) and hexose monophosphate shunt activity (4). One class of important cancer chemotherapeutic agents, the vinca alkaloids, is known to impair microtubule function (5). Since microtubules are involved in certain aspects of neutrophil function (6), we decided to examine the effects of vinca alkaloids on neutrophils.

The effects of three vinca alkaloids (vinblastine, vincristine, and vindesine) on phagocytosis and neutrophil iodination were studied. Although neutrophil iodination is an important neutrophil bactericidal mechanism, and an ideal function test since it is a result of ingestion, degranulation, and H_2O_2 production, there have been no previous reports describing the effects of these drugs on this system.

MATERIALS AND METHODS

Preparation of Neutrophil Suspensions

Neutrophils were prepared by a modification of the method of Dewar (7). Peripheral blood (20 ml) was collected from normal volunteers into 2 × 10-ml heparinized plastic tubes (100 µl Li heparin; Johns Products). The heparinized blood was diluted 2:3 with phosphate-buffered saline (PBS), and layered onto 10 ml of Histopaque 1077 (Sigma) in 25-ml plastic tubes (Filtrona Plastics) which were centrifuged for 30 mins at 400 × *g*. Supernatants were removed and the pellets containing neutrophils and erythrocytes were washed and resuspended in PBS. Dextran T500 in saline (6 ml; 0.040 g/ml) was added to the tubes which were then gently vortexed. The erythrocytes were allowed to sediment for 30 mins at 4°C, after which time the neutrophil-rich supernatants were collected and washed in PBS. Remaining erythrocytes were removed by hypertonic lysis. The final cell concentration of the neutrophil suspension in PBS was adjusted to 10^7 cells/ml. Vi-

[1]Received Apr 27, 1981; revised Aug 21, 1981; accepted Sept 2, 1981.

[2]Supported by the National Health and Medical Research Council of Australia and the David Collins Leukemia Foundation of Tasmania.

[3]Vindesine was supplied by Lilly Industries Pty Ltd, Sydney, Australia.

[4]Department of Medicine, University of Tasmania, Hobart, Australia.

[5]We thank Dr. Roger Connolly of the Department of Nuclear Medicine, Royal Hobart Hospital, for the use of a gamma counter.

*Reprint requests to: Dr. R. M. Lowenthal, Department of Medicine, University of Tasmania, 43 Collins St, Hobart, Tasmania, 7000 Australia.

ability as determined by eosin dye exclusion was always > 97%.

Organisms

Cultures of *Staphylococcus aureus*, *Candida albicans*, and *Saccharomyces cerevisiae* were kindly supplied by the Microbiology Department of the University of Tasmania. The organisms were heat-killed by incubation at 60°C for 1 hr, then suspended in PBS. The *C. albicans* and *Saccharomyces cerevisiae* suspensions were adjusted to 0.8 OD at 540 nm using a cuvette with a 1-cm light path. The *Staphylococcus aureus* suspension was adjusted to 0.9 OD.

Preparation of Drug Solutions

Drugs were freshly prepared as 1 mM solutions in water, and successive dilutions were made to obtain concentrations of 33, 3.3, 0.3, and 0.03 μM. These solutions were used in the test mixtures.

Measurement of Phagocytic Index

The phagocytosis of *Saccharomyces cerevisiae* was measured essentially according to the method of Hosking et al (8), except that the smears were stained with May-Grünwald-Giemsa, and the number of yeast organisms ingested in 50 neutrophils was counted. The phagocytic index is the mean number of yeast organisms ingested per neutrophil. The effect of drugs on phagocytosis was tested by replacing 24 μl of the glucose solution with a drug solution at the appropriate concentration to give a final concentration of 10^{-6}, 10^{-7}, 10^{-8}, or 10^{-9} M. All tests were carried out in duplicate. The mean phagocytic index for each drug sample was expressed as a percent of the mean control value, taken as 100%.

Measurement of Neutrophil Iodination

For these measurements, the basic methodology of Hosking et al (8) was employed. The iodination of *Staphylococcus aureus* and of *C. albicans* was tested. All tests were carried out in triplicate in polystyrene tubes (Johns Products). The control tube contained 300 μl of glucose solution (40 mg of glucose/100 ml of PBS), 100 μl of *Staphylococcus aureus* or *C. albicans* suspension, 25 μl of pooled human serum, 100 μl of an ^{125}I solution in PBS (0.6 microcurie/ml; activity > 14 millicuries/μg), and 100 μl of the neutrophil suspension. For the samples testing drug effects, 20 μl of the glucose solution was replaced with a drug solution at the appropriate concentration to give a final concentration of 10^{-6}, 10^{-7}, 10^{-8}, or 10^{-9} M. This range covers the concentrations of vinblastine and vincristine achieved in vivo during cancer chemotherapy (9). There is insufficient information available about vindesine to determine its concentration in vivo. The tubes were vortexed gently, then incubated for 60 mins at 37°C in a shaking water bath. The reaction was terminated and worked up as described by Hosking et al (8). The mean of the three controls for each experiment was taken as 100% iodination. The average of the counts for each drug sample was expressed as a percent of the control value.

Statistics

Student's t test was used to assess the significance of values for the drug-treated samples.

RESULTS

Effects of Drugs on Phagocytosis

The phagocytic index of neutrophils after 10- and 60-min incubation periods with each of the three vinca alkaloids, at the four different concentrations, is shown in table 1. When the neutrophils were allowed to phagocytose for only 10 mins no difference in phagocytosis was observed with any of the drugs when compared with the control. This was also the case with all concentrations of vinblastine and vindesine when phagocytosis was allowed to proceed for 60 mins. However, dose-dependent reductions in phagocytosis were apparent with vincristine at concentrations of 10^{-6} M ($P < 0.025$), 10^{-7} M ($P < 0.025$), and 10^{-8} M ($P < 0.050$).

Effects of Drugs on Iodination

The iodination of *Staphylococcus aureus* and *C. albicans* by drug-treated neutrophils, expressed as percents of the controls, is shown in table 2. Reductions in iodination were observed with a concentration of 10^{-6} M vinblastine and vincristine. A slight enhancement was recorded with 10^{-6} M vindesine. However, each drug affected the iodination of only one particle. Vinblastine and vincristine reduced the iodination of *C. albicans* when compared with the control, whereas vindesine exerted its effect on the iodination of *Staphylococcus aureus*. The lower concentrations (10^{-7}, 10^{-8}, and 10^{-9} M) of the three drugs produced no changes in iodination of either of the particles.

TABLE 1.—Effect of 3 vinca alkaloids on the phagocytic index of neutrophils in vitro*

Drug	Concentration (M)	Phagocytic index† at 2 incubation times	
		10 mins	60 mins
Vinblastine	10^{-6}	99 ± 0.7	92 ± 5.5
	10^{-7}	102 ± 2.0	101 ± 3.5
	10^{-8}	94 ± 4.3	104 ± 2.2
	10^{-9}	101 ± 2.3	103 ± 3.8
Vincristine	10^{-6}	100 ± 2.0	87 ± 2.3[a]
	10^{-7}	106 ± 2.7	89 ± 1.5[a]
	10^{-8}	101 ± 4.0	94 ± 1.3[b]
	10^{-9}	95 ± 3.6	98 ± 2.2
Vindesine	10^{-6}	97 ± 2.0	100 ± 1.3
	10^{-7}	96 ± 3.0	93 ± 3.0
	10^{-8}	97 ± 2.7	96 ± 4.4
	10^{-9}	106 ± 4.0	97 ± 2.2

*All observations are the results of at least 3 experiments.

†Results are expressed as % of control value ± SEM. Experimental values were not significantly different from the controls except where indicated by (a) $P < 0.025$ or (b) $P < 0.050$.

TABLE 2.—Effect of 3 vinca alkaloids on neutrophil index iodination in vitro*

Drug	Concentration (M)	Iodination† using 2 particles	
		Staphylococcus aureus	*C. albicans*
Vinblastine	10^{-6}	101 ± 0.9	89 ± 2.5[b]
	10^{-7}	105 ± 3.5	96 ± 2.0
	10^{-8}	108 ± 3.4	104 ± 3.5
	10^{-9}	106 ± 3.0	104 ± 1.0
Vincristine	10^{-6}	101 ± 2.0	93 ± 1.7[b]
	10^{-7}	105 ± 1.0	97 ± 1.2
	10^{-8}	102 ± 1.8	101 ± 0.4
	10^{-9}	104 ± 3.5	103 ± 2.0
Vindesine	10^{-6}	104 ± 0.5[a]	97 ± 4.5
	10^{-7}	99 ± 1.6	96 ± 2.9
	10^{-8}	100 ± 2.5	102 ± 3.2
	10^{-9}	97 ± 3.6	102 ± 1.8

*All observations are the results of at least 3 experiments.

†Results are expressed as % of control value ± SEM. Differences between control and experimental values were not significant except where indicated by (a) $P < 0.005$ or (b) $P < 0.025$.

DISCUSSION

This study was designed to determine whether vinblastine and vincristine, two important clinical oncolytic agents, and vindesine, a drug which is gaining increasing interest in cancer chemotherapy, had any effect on two neutrophil functions directly involved in protection of the host from infections, ie, neutrophil phagocytosis and iodination.

We found slight but statistically significant reductions in phagocytosis after 60-min incubations with vincristine, but not vinblastine and vindesine, at concentrations of 10^{-6}, 10^{-7}, and 10^{-8} M (table 1). The possibility that the *rate* of phagocytosis may have been decreased in the presence of these three vinca alkaloids was also considered. Measurements of the phagocytic rate must be made during the period where particle uptake is maximal and proportional to the period of incubation (10). This period is > 10 mins for ingestion of *Saccharomyces cerevisiae* by neutrophils,[6] and the phagocytic index was measured after 10-min incubations. In these

[6]Pereira HA. Unpublished results.

cases no differences were seen between drug-treated and control neutrophils (table 1). Thus, vincristine, at concentrations of 10^{-6}, 10^{-7}, and 10^{-8} M, did not affect the phagocytic *rate* of neutrophils but decreased their phagocytic *capacity*.

A limited number of reports have described the effects of vinblastine and vincristine on neutrophil phagocytosis. Whittaker et al (2), using only above-therapeutic concentrations and a light microscopic method, observed a depression in the phagocytosis of *C. albicans* by these agents. In contrast, Malawista (11), who used vinblastine at high concentrations (2.5×10^{-5} M) but the less sensitive plating-out method, found no effect on the phagocytosis of *Staphylococcus aureus*. These apparently inconsistent conclusions may be explained by variations in the methods employed and by the use of different particles (12,13).

In our other set of experiments, we found a reduction of iodination of *C. albicans* by neutrophils in the presence of 10^{-6} M vinblastine and vincristine, and a slight enhancement of iodination of *Staphylococcus aureus* by 10^{-6} M vindesine (table 2). The findings emphasize that the effect of a drug on cell function will vary with the use of different particles. Relatively little is known about vindesine and its different behavior may suggest that it acts differently from vinblastine and vincristine. In all instances the effects were slight, and even though they were statistically significant, the biologic importance would be negligible. Furthermore, the concentration at which vinblastine and vincristine produced an inhibitory effect (10^{-6} M) is in excess of that achieved in vivo during cancer chemotherapy (9). At such concentrations (10^{-7}–10^{-8} M), none of the drugs had any effect on iodination of either particle.

Since iodination is dependent on phagocytosis, one might expect the reduction in the phagocytic capacity of neutrophils produced by 10^{-7} and 10^{-8} M vincristine (table 1) to result in a reduction in iodination too. However, this was not the case. The apparent inconsistency is resolved when it is recalled that the phagocytic rate was unaffected. So in vitro alterations in phagocytic capacity alone may not be a sensitive predictor of clinically important abnormalities in neutrophil iodination.

We conclude that therapeutic concentrations of vinblastine, vincristine, and vindesine do not impair neutrophil functions that are important in the protection of the host against infection.

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Studies on the Pharmacokinetics and Metabolism of *cis*-Dichloro-*trans*-dihydroxy-bis-isopropylamine platinum(IV) in the Dog [1,2]

Lakshmi Pendyala,* J. Wayne Cowens, and Patrick J. Creaven [3,4]

SUMMARY

***cis*-Dichloro-*trans*-dihydroxy-bis-isopropylamine platinum(IV) (CHIP), a new antineoplastic platinum compound, was administered iv to dogs at a dose of 10 mg/kg. Thin-layer chromatography and high-pressure liquid chromatography (HPLC) were used to evaluate the pharmacokinetics of total Pt and of unchanged drug separated from the metabolites in urine. Both chromatographic procedures indicated a half-life of 0.3–0.5 hr for the unchanged CHIP (based on urinary excretion rates). Decay of total Pt after administration of CHIP was biphasic, with an α-half-life of 0.6 hr and a β-half-life of 39.4 hrs. The long β-half-life thus appears to be due to the retention of metabolites. Unchanged CHIP was separated from two groups of more polar Pt-containing metabolites by HPLC, as well as from another less polar Pt-containing complex that is found in urine of untreated dogs to which CHIP was added. After the administration of CHIP, with the simultaneous decline in its level, the proportion of the very polar Pt-containing metabolite(s) accumulated in the dog urine. Unlike cisplatin, CHIP did not bind to plasma proteins in vitro.**

[Cancer Treat Rep 66:509–516, 1982]

Since the original observation of the antitumor activity of cisplatin against Sarcoma 180 in mice (1), a substantial body of information has been obtained from clinical studies on the wide spectrum of activity of this drug, both as a single agent and in combination with other drugs, against human tumors (2). Since administration of cisplatin often leads to irreversible and occasionally fatal nephrotoxicity (3), there has been a considerable interest in the study of second-generation Pt compounds which retain antitumor activity but do not have significant renal toxicity. Several hundred analogs of cisplatin have been screened against the adj/PC6 plasmacytoma and from these a number have been identified which have a therapeutic index greater than or equal to that of cisplatin (4–6). Most of these have been complexes of divalent Pt, like cisplatin, but a few have been complexes of quadrivalent Pt (Pt IV). One such Pt IV complex is *cis*-dichloro-*trans*-dihydroxy-bis-isopropylamine platinum(IV) (CHIP) (fig 1), which in addition to activity against adj/PC6 plasmacytoma shows activity against L1210 leukemia (4,7). CHIP is approximately five times more water-soluble than cisplatin (solubilities: CHIP, 44.1 mM; cisplatin, 8.9 mM), and preliminary studies in mice suggested that the compound is less nephrotoxic than cisplatin, when tested at or above the dose producing maximum survival (5,7). For this reason, CHIP underwent preclinical drug development at this institute, preparatory to being introduced into initial clinical trial. At equitoxic doses, CHIP was found to be more active than cisplatin in inhibiting

[1]Received May 7, 1981; revised Aug 12, 1981; accepted Aug 18, 1981.

[2]Supported in part by Public Health Service grant CA-21071 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Departments of Clinical Pharmacology and Therapeutics (*L. Pendyala* and *P. J. Creaven*) and Experimental Therapeutics (*J. W. Cowens*), New York State Department of Health, Roswell Park Memorial Institute, Buffalo.

[4]The technical assistance of Miss Mary Bajzik is gratefully acknowledged.

**Reprint requests to:* Dr. Lakshmi Pendyala, Department of Clinical Pharmacology and Therapeutics, New York State Department of Health, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263.

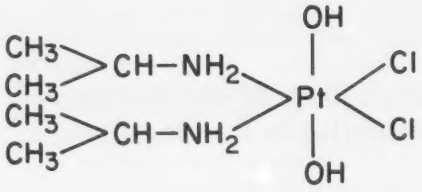

FIGURE 1.—Structure of CHIP.

the growth of Walker 256 carcinosarcoma and Murphy-Sturm lymphosarcoma and produced a greater increase in the lifespan of mice with L1210 leukemia (8). The dose-limiting toxicity of the compound in the rat and the dog was found to be myelosuppressive rather than renal (8,9). We report here studies of the pharmacokinetics of CHIP in the dog. Preliminary reports of some of these studies have appeared previously (10,11).

MATERIALS AND METHODS

Dog experiments.—Adult male beagle dogs (9–12 kg) were given 10 mg/kg of CHIP (supplied by Johnson Matthey Co, England[5]) in 0.9% saline iv and were kept in metabolism cages. Blood samples were collected in heparinized tubes at timed intervals up to 48–72 hrs and centrifuged immediately to obtain plasma. Urine samples were collected up to 7–9 days after CHIP administration. The urine samples of the first day were obtained by catheterization and the later samples were collected from the metabolism cage. All of the samples were stored frozen until analysis.

Pt estimations.—The total Pt in plasma and urine samples was estimated by flameless atomic absorption spectrophotometry (FAAS) using a Perkin Elmer Model 403 atomic absorption spectrophotometer equipped with a Model 2100 graphite furnace. A three-stage temperature program with 50-sec drying, 50-sec charring, and 20-sec atomization was used. Argon was used as the inert purge gas. Urine samples (20–50 μl) were injected directly into the furnace, while plasma samples were predigested with nitric acid and redissolved in 1 N HCl. An aliquot (20–50 μl) of this digest was then injected into the furnace. To allow for matrix effects, standard curves were prepared using an identical procedure. The absorbance of identical volumes of samples and standards were compared to obtain the Pt concentrations. Values reported were the average of two to four separate Pt determinations for each sample. The detection limit for Pt under these conditions was 1 ng.

Thin-layer chromatography (TLC).—Dog urine samples containing about 5–10 μg of Pt were spotted on Whatman PLK5 Linear K silica gel-coated glass plates (20 × 20 cm; 1000 μm thick) and developed in 9:1 acetone:H_2O, until the solvent front was 0.5 inch from the top. The Pt-containing spots on the plate were visualized by spraying the plate, after drying, with a mixture of stannous chloride (5.6%) and potassium iodide (0.2%) in 1.2 N HCl (12). The R_f value for CHIP was 0.75. For quantitation of Pt, successive 1-cm segments of coating on the glass plate from the origin to the solvent front were scraped off into conical glass centrifuge tubes and extracted with 0.3–0.5 ml of 1 N HCl. The extract was centrifuged and 20–50 μl of the clear supernatant was injected into the graphite furnace for Pt estimation. Standard curves were prepared from extracts of known amounts of Pt spotted on identically treated chromatography plates.

High-pressure liquid chromatography (HPLC).—The HPLC system consisted of Waters Associates Model 6000A pumps, a U6K injector, a Model 440 UV detector, and a Model 660 solvent programmer. The separation of CHIP and metabolites in urine samples was carried out on a μBondapak C18 column (Waters Associates; 3.9-mm ID × 30 cm) using water (pH 4.0) to methanol gradient elution (curve 7 on the solvent programmer; 40-min program). The mobile-phase solvents were filtered through 0.45-μm Millipore membrane filters and thoroughly vacuum-degassed. The flow rate was 1.5 ml/min. Since ultraviolet absorption of CHIP is negligible, fractions of the column eluate were collected and Pt was estimated by FAAS. The retention volume for CHIP was 20 ml under these conditions. To protect the column from contamination, a quantitative extraction procedure for Pt-containing compounds in urine was developed. Ultrafiltered urine (0.5–3 ml) was passed through C18 Seppak cartridges (Waters Associates) and prewetted with 2 ml of acetonitrile and 6 ml of water. The cartridge was then eluted with successive washes of 30%, 70%, and 100% methanol and the eluates were pooled with the void volume and evaporated to dryness by lyophilization. The residue was redissolved in 0.2–0.5 ml of water, and 50–150 μl of this concentrate was then injected onto the HPLC for the separation of CHIP from metabolites. The recovery of Pt is 100% by this procedure.

In vitro plasma binding studies.—The plasma protein binding of CHIP in comparison to cisplatin was investigated using ultrafiltration (13). CHIP (1–5 μg/ml) or cisplatin (1–5 μg/ml) was added to freshly prepared plasma and incubated at 37°C in

[5]Sponsor of CHIP in the US is: Bristol Laboratories, Syracuse, NY.

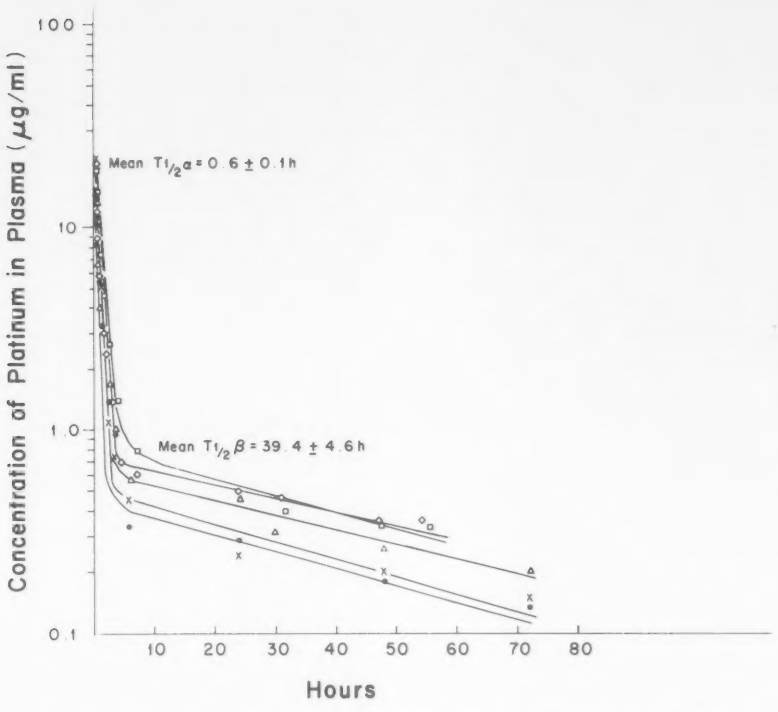

FIGURE 2.—Plasma decay of Pt in the dog after administration of 10 mg/kg iv of CHIP. Each symbol represents a different dog.

the dark for a period up to 48 hrs. Aliquots were taken at timed intervals and ultrafiltered through Amicon CF50A filters (50,000 molecular weight cut-off) for 20 mins at 1000 × *g* in a refrigerated clinical centrifuge. Concentrations of Pt in the plasma and the ultrafiltrate were determined by FAAS.

RESULTS

Plasma decay of total Pt.—The decline in plasma concentration of total Pt following the iv administration of CHIP (10 mg/kg) to dogs, as measured by FAAS analysis of plasma samples, is shown in figure 2. The figure represents data from five dogs. The decay of Pt in plasma is biphasic. The concentration of Pt in the first phase declined very rapidly, with an estimated α-half-life of 0.6 ± 0.1 hr. The decline in the Pt concentration in the second phase was much slower, with a mean β-half-life of 39.4 ± 4.6 hrs.

Urinary excretion of Pt.—The urinary excretion of Pt with time after administering CHIP is shown in figure 3 as a percent of administered dose. Pt is excreted quite rapidly in the first several hours after drug administration, but after 24 hrs very little further Pt is excreted. There was a considerable variability in the percent of the total Pt excreted in five dogs; the excretion at 24 hrs was 45%–72% of the total dose administered.

Urinary excretion of unchanged CHIP.—The urinary excretion of unchanged CHIP and its metabolic products was studied using TLC and HPLC. Both methods indicated rapid excretion of unchanged CHIP. In figure 4, the separation of unchanged CHIP from the Pt-containing metabolites by TLC is shown in successive samples of urine collected from one dog that had received CHIP. As seen in this figure, the proportion of unchanged CHIP in each urine sample declined rapidly while that of other Pt-containing complexes increased. The de-

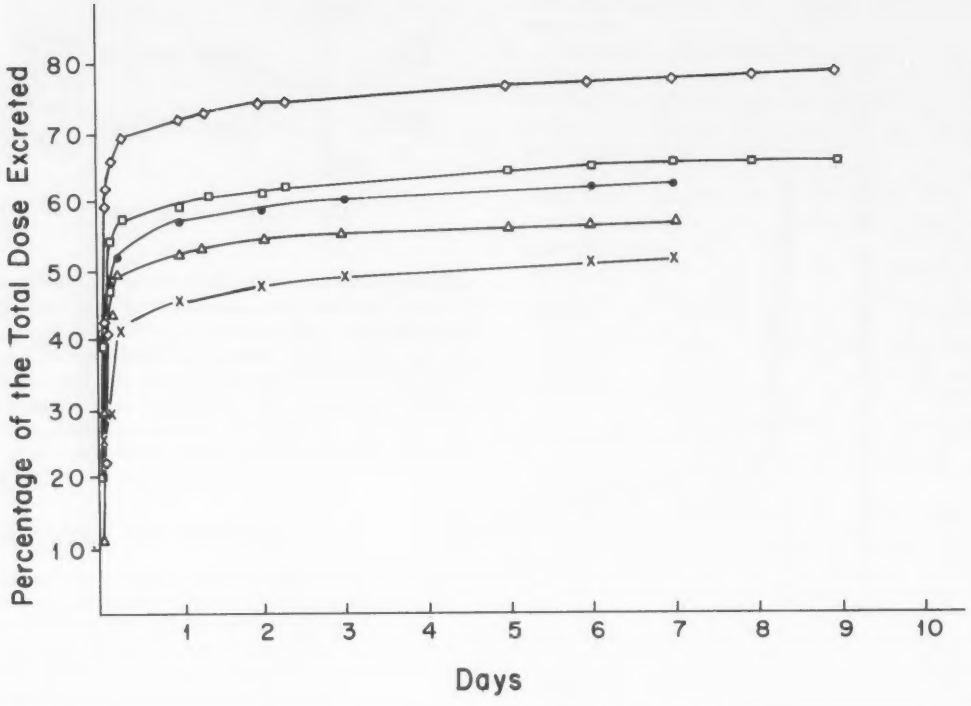

FIGURE 3.—Urinary excretion of Pt in the dog after administration of 10 mg/kg iv of CHIP. Each symbol represents a different dog.

cline in the urinary levels of unchanged CHIP in three dogs, as determined by TLC, is shown in figure 5. The successive urine samples from the same dog shown in figure 4 were analyzed for unchanged CHIP by HPLC (fig 6). This method allowed the separation of CHIP from two other groups of more polar Pt-containing metabolites present in the dog urine samples obtained after CHIP administration. Again, this method confirmed the rapid decline in the level of unchanged CHIP. A simultaneous increase in the relative proportion of polar Pt-containing metabolites occurred with the decline in unchanged CHIP (fig 6). In two other dogs studied, an identical pattern was seen with respect to the decline in the proportion of CHIP and increase in that of the polar metabolites. The urinary excretion rates of unchanged CHIP were calculated from the proportion of unchanged CHIP found in each of the urine samples by TLC and HPLC. The apparent plasma half-life of unchanged CHIP was then calculated from the urinary excretion rates of unchanged CHIP and found to be 0.3–0.5 hr (14). During the chromatography of dog urine samples, it was found that CHIP can produce Pt-containing breakdown products if it is left in the urine for several hours at room temperature. These breakdown products of CHIP had longer retention on the C18 column compared to CHIP and thus appear to be less polar and distinct from the Pt-containing metabolites found in the urine of dogs treated with CHIP (fig 7).

Protein binding of CHIP in vitro.—In in vitro experiments comparing the plasma protein binding of CHIP and cisplatin, significant differences were found between the two compounds (fig 8). As determined by ultrafiltration (13), we found that even at 48 hrs of incubation at 37°C, CHIP was completely recovered in the ultrafiltrate, while cisplatin showed a typical, biphasic decreased recovery as reported earlier (13).

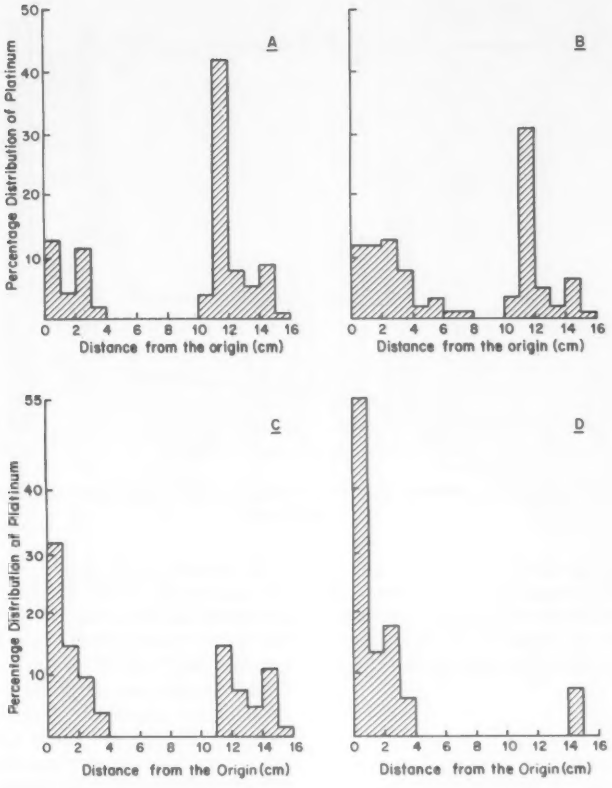

FIGURE 4.—Separation of CHIP from other Pt-containing compounds in dog urine using TLC. A–D = dog urine samples collected at successive times after administration of CHIP (A = 0.2 hr, B = 1.2 hrs, C = 2.7 hrs, D = 3.8 hrs). Pt in segments 10–13 = unchanged CHIP. Each bar = % of total Pt on the chromatogram estimated in that 1-cm section by FAAS.

DISCUSSION

CHIP is one of a number of cisplatin analogs that are being evaluated for their antitumor, toxic, and biochemical properties (15–20). It is of considerable interest that its dose-limiting toxicity was shown to be myelosuppression, and little or no renal toxicity has been observed (8,9). The pharmacokinetic studies reported in this paper indicate that the concentration of Pt in plasma after CHIP administration declined in a biphasic manner, as reported for cisplatin. However, the β-half-life of Pt after CHIP was only 39.4 hrs, which is considerably shorter than the β-half-life of 120 hrs reported for Pt after cisplatin administration in the dog (13). A much shorter β-half-life for Pt after CHIP compared to cisplatin was also observed in the rat (9). This faster decay of total Pt may be one of the reasons for the decreased nephrotoxicity observed with this compound as compared with cisplatin. The urinary excretion of total Pt after CHIP administration was in a range similar to that reported for cisplatin in the dog (21).

Although failure to recover total Pt in the urine prevents calculation of accurate half-lives from the data on urinary excretion rates, an approximate biologic half-life of CHIP could be calculated. This gave a value of 0.3–0.5 hr for CHIP by both TLC and HPLC techniques, indicating a dramatic difference between the pharmacokinetics of the unchanged drug and that of total Pt. We could detect unchanged CHIP up to approximately 7 hrs using

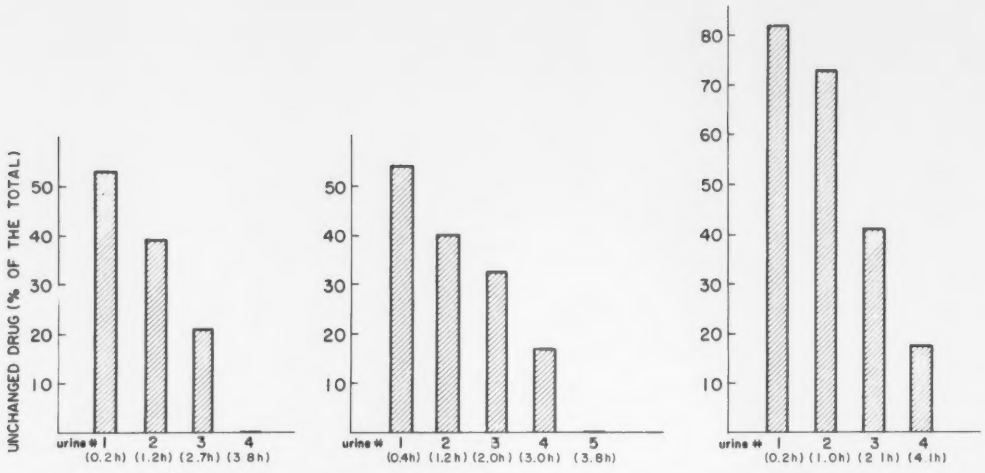

FIGURE 5.—Unchanged CHIP in dog urine, shown as % of total Pt at the various urine collection times in 3 dogs as estimated by TLC and FAAS.

the HPLC methodology in all dogs studied. Thus, it appears that the long β-half-life of Pt observed after CHIP must be due to the retention of metabolites. A short half-life for cisplatin in the plasma of patients treated with this drug was reported recently (22,23).

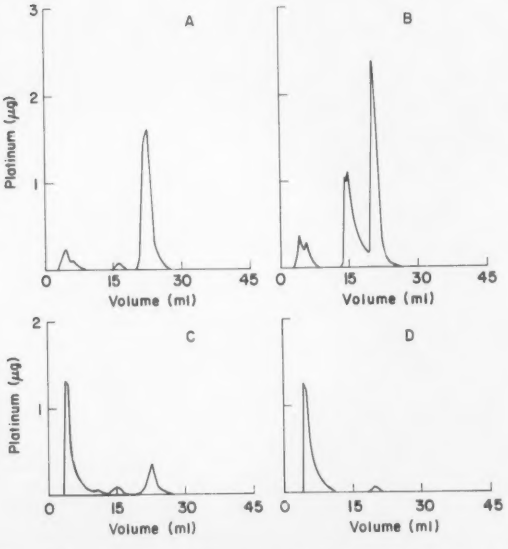

FIGURE 6.—Pt-containing compounds in urine at 0.2 hr (A), 1.2 hrs (B), 2.7 hrs (C), and 3.8 hrs (D) after CHIP injection, as measured by HPLC and FAAS. Retention volume of CHIP is 20 ml.

It was observed that with the decline in the level of unchanged CHIP the level of polar metabolites increased in the urine. It is of interest to note that the major metabolites of CHIP in the urine of dogs treated with CHIP did not include the less polar CHIP breakdown products observed when CHIP is dissolved in urine and allowed to stand. Some urine samples did contain these CHIP breakdown products, but their concentration never exceeded 2%–4% of the total Pt. It is possible that these small amounts may have formed during the processing of the urine. The more polar nature of urinary metabolites indicates the possible involvement of the Cl and OH groups in reactions leading to the genera-

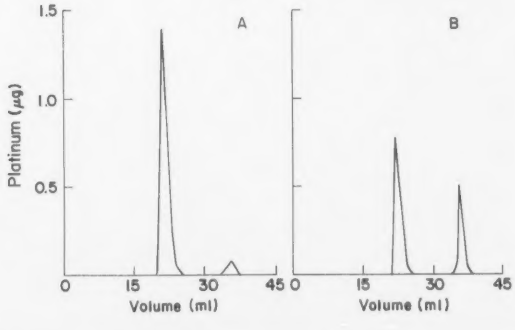

FIGURE 7.—HPLC separation of CHIP from the Pt-containing breakdown products found in urine when CHIP was added to dog urine. A. CHIP in urine (freshly prepared); B. CHIP in urine (remained at room temperature for 8 hrs).

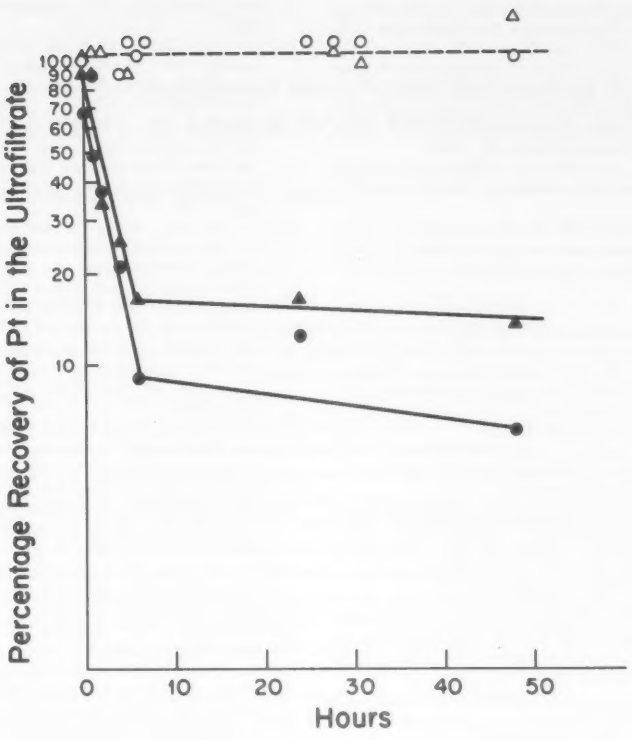

FIGURE 8.—Recovery of Pt in the plasma ultrafiltrate at various sampling times after incubating CHIP (○ = 1 μg/ml; △ = 5 μg/ml), or cisplatin (● = 1 μg/ml; ▲ = 5 μg/ml) in plasma up to 48 hrs at 37°C.

tion of these new compounds, if the metabolism of CHIP parallels that of cisplatin (24). Alternatively, they may have been generated by the loss of isopropyl groups from CHIP. Using conventional cation exchange chromatography, LeRoy et al (25) have recently separated cisplatin from two other Pt complexes in the urine of patients treated with cisplatin, and demonstrated that one of them is also present in in vitro incubations of cisplatin in control urine.

CHIP and cisplatin differ with respect to their plasma protein binding properties. CHIP neither binds to plasma proteins nor seems to form any products in in vitro incubations with plasma that bind to plasma proteins (fig 8). On the other hand, cisplatin exhibits an increased binding to plasma proteins after in vitro incubation, which has been attributed to the reactivity of cisplatin or its aquated form in biologic fluids (26). Some of the small molecular weight biotransformation products so formed were separated by HPLC (26). Although CHIP does not bind to plasma proteins, we have no information as yet about the possible protein binding of its metabolites, and further experiments are necessary to understand the long β-half-life of 39.4 hrs.

The development of a specific chromatographic method (HPLC) has enabled us to separate CHIP from its metabolites and breakdown products in urine. Studies are underway to increase the sensitivity of this method to the point where it can be used for direct assay of unchanged CHIP in plasma and also for the study of the nature of the metabolites and breakdown products.

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Enhanced Lymph Node Uptake of Melphalan Following Liposomal Entrapment and Effects on Lymph Node Metastasis in Rats[1]

Juneji Khato, Elaine R. Priester, and Susan M. Sieber[2,3,*]

SUMMARY

The effect of liposomal entrapment of melphalan (MPL) on its tissue distribution and activity against experimental lymph node metastasis was evaluated in rats. Neutral small liposomes containing MPL and the fluorescent marker carboxyfluorescein (CF) were prepared by sonication using phosphatidylcholine (PC) and cholesterol (molar ratio, 2:1), along with tracer amounts of [^{14}C]MPL and [^{3}H]PC. Unlike CF, MPL appears to associate with the lipid phase of liposomes; entrapped MPL was not hydrolyzed and was gradually released from liposomes during incubation at 37°C. Free [^{14}C]MPL injected sc into the thigh of rats was rapidly cleared from the injection site, with only 0.6% of the ^{14}C activity remaining after 2 hours. The concentration of MPL in the ipsilateral inguinal lymph nodes showed a transient increase over that in contralateral nodes at 0.5 hour, although MPL concentrations were similar in ipsilateral and contralateral nodes at 1 hour. After sc injection of [^{14}C]MPL/[^{3}H]PC-liposomes, about 4% of the ^{14}C activity was present at the injection site at 2 hours after treatment. The concentration of PC equivalents in ipsilateral lymph nodes was at least 50 times higher than that in plasma, lung, or contralateral lymph nodes at all time intervals examined. Similarly, liposomal entrapment of MPL enhanced its uptake by ipsilateral lymph nodes; this increase in lymph node MPL concentration was sustained for at least 24 hours after dosing, at which time ipsilateral nodes contained 20-fold and tenfold higher MPL levels than were present in plasma and contralateral nodes, respectively. Rats were inoculated sc in the calf with a tumor brei (mammary adenocarcinoma 13762), the leg was amputated 10 days after inoculation, and animals were treated on Day 13 with free or liposome-entrapped MPL injected sc into the thigh. Liposome-entrapped MPL produced a greater reduction in the weight of lymph node metastases as compared with free MPL. Thus, when MPL was entrapped in liposomes, a dose of 0.125 mg/kg reduced the weight of lymph node metastases to about 50% that in controls. A dose of 0.5–1.0 mg/kg was required to exert an equivalent effect on lymph node tumor growth when free MPL was administered. These results suggest that the interstitial injection of liposomes containing MPL may be useful for the postoperative treatment of lymph node metastases.

[Cancer Treat Rep 66:517–527, 1982]

[1]Received May 19, 1981; revised Sept 18, 1981; accepted Sept 29, 1981.

[2]Laboratory of Chemical Pharmacology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

[3]We thank Drs. Richard H. Adamson, Tomowo Kobayashi, and David T. Vistica for their helpful discussions.

Reprint requests to: Susan M. Sieber, PhD, Laboratory of Chemical Pharmacology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bldg 37, Rm 5D09, National Institutes of Health, Bethesda, MD 20205.

The lymphatics are known to play an important role in the removal of high molecular weight substances from the interstitial space (1), and a variety of particles (rbcs, chylomicra, carbon, ferritin, etc) have been observed passing through normal and injured lymphatic capillary endothelia in a number of sites in the body (6). These observations provided the basis for attempts at targeting antitumor agents to lymph nodes containing metastases by binding such agents to high molecular weight substances (14) or administering them in lipid emulsions (23). However, liposomal entrapment might provide an alternative approach to targeting drugs to lymph nodes via lymphatic capillaries because of the particulate nature of liposomes (20–22). A potential advantage of liposomes as carriers of antitumor agents is that they can be prepared from a variety of chemically purified and biodegradable phospholipids by methods that yield liposomes with reproducible characteristics related to size, surface charge, drug entrapment rate, stability, and permeability. In recent years, numerous studies on the potential utility of liposomes as carriers for various biologic materials have been reported. For example, cytarabine entrapped in the aqueous phase of liposomes and administered ip to mice bearing the ascitic form of L1210 leukemia has been shown to be more effective than free drug in prolonging survival time (12,17,18). However, only a few reports have appeared which describe lymph node uptake following interstitial injection of liposomes containing anticancer agents (20–22), and little is known as to the effects of liposome-entrapped antitumor agents on lymph node metastasis.

The purpose of the present study was to assess the feasibility of employing liposomal entrapment as a means of directing antitumor agents to regional lymph nodes. Melphalan (MPL) was selected as the agent to be entrapped because of its amphiphilic nature and its activity against a variety of tumors including mammary cancer (5). The distribution and lymph node uptake of MPL entrapped in liposomes and administered by sc injection were compared with those of free MPL; in addition, the in vivo effects on lymph node metastasis were evaluated using the MPL-sensitive metastatic mammary adenocarcinoma 13762 in rats.

MATERIALS AND METHODS

Chemicals.—Egg phosphatidylcholine (PC) (in ethanol) was purchased from Grand Island Biological Co, NY; cholesterol (CH) was obtained from Sigma Chemical Co, St. Louis, MO; MPL was supplied by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD; L-α-dipalmitoyl(2-palmitoyl-9,10-^{3}H)PC (13 curies/mmol) was purchased from Applied Science Laboratories Inc, State College, PA; and [chloroethyl-^{14}C]MPL (13.0 millicuries/mmol) was supplied by the Pharmaceutical Resources Branch, Division of Cancer Treatment, National Cancer Institute. Unlabeled PC, CH, and MPL each migrated as a single band when subjected to silica gel thin-layer chromatography (TLC) using chloroform:methanol:water (65:25:4) as the solvent system. The radiochemical purity of [^{3}H]PC and [^{14}C]MPL was > 97% as determined by TLC in the above system. The extent to which MPL was hydrolyzed during sonication was determined by TLC on cellulose plates using isopropanol:formic acid:water (6.5:0.11:3.4) as the solvent system. CF was purchased from Eastman Kodak Co, Rochester, NY, and was purified by recrystallization. Phosphate-buffered saline (PBS) (pH 7.4) contained NaCl (137 mM), KCl (2.6 mM), Na_2HPO_4 (6.4 mM), and KH_2PO_4 (1.4 mM), and it was free of Ca^{++} and Mg^{++}. Prior to its use in the preparation of liposomes, PBS was filtered through 0.2-μ filters to avoid particulate contamination.

Preparation of liposomes containing MPL.—Neutral small liposomes were prepared by sonication of a thin lipid film with an aqueous solution. MPL was either codeposited with lipid (lipid phase liposomes) or dissolved in aqueous solution (aqueous phase liposomes) before sonication. Lipid phase liposomes were prepared as follows. An ethanol solution containing 160 μmols of PC, 80 μmols of CH, and 24 μmols of MPL, along with tracer amounts of [^{3}H]PC and [^{14}C]MPL, was placed in a 50-ml round-bottom flask. The solvent was removed using a rotary evaporator for 1 hour under reduced pressure at 25°C. The flask was kept in vacuo at room temperature using a vacuum pump for an additional 1 hour to ensure the complete removal of the solvent. Five milliliters of a 1/10 dilution of PBS containing CF (20 mM) was added to the flask, and the mixture was sonicated for approximately 1 hour at 25°C using the microtip of a Heat Systems Sonicator from Ultrasonic, Inc, Plainview, NY. Clearing of the cloudy suspension signaled the formation of small vesicles. The sonicate was subjected to Sephadex G-25M (Pharmacia Fine Chemicals AB, Uppsala, Sweden) column chromatography to remove extravesicular MPL and CF. The liposome suspension obtained was kept on ice and was used within a few hours after preparation. Aqueous phase liposomes were prepared by sonicating the lipid film with an aqueous solution containing MPL and CF. "Control" liposomes were prepared in an identical manner but without the addition of MPL to the system.

Determination of CF in liposomes.—CF is a fluorescent dye which was incorporated into MPL-liposomes as a marker for the aqueous compartment; it also proved to be a useful visual marker for readily distinguishing liposome fractions eluted during Sephadex column chromatography from other fractions containing free MPL and free CF. Suspensions of liposomes were diluted with PBS containing 0.1% Triton X-100, and the concentration of CF in diluted aliquots was determined using an Aminco Bowman spectrophotofluorometer (Model SPFl, American Instrument Co, Silver Spring, MD) equipped with a polarizing filter to reduce light scatter. Measurements were made using an excitation wavelength of 490 nm and an emission wavelength of 520 nm (25).

In vitro studies of the release of [^{14}C]MPL and CF from liposomes.—Aliquots of freshly prepared liposomes were added to cellulose dialysis bags of 0.7-cm diameter (nominal molecular weight cut-off, 12,000). The bags were dialyzed against 150 volumes of PBS at 4°C and 37°C, with a change of dialysate at 0.5, 1, 2, 4, 6, and 8 hours. The amount of ^{14}C activity and CF remaining in the dialysis bags was determined at intervals. In control experiments, it was shown that free MPL and CF dialyzed completely from the bag within 2 hours at 4°C.

Tissue distribution studies after sc injection of free or liposome-entrapped [^{14}C]MPL.—Female Fischer 344 rats, 35–42 days of age, were obtained from Charles River Laboratories (Wilmington, MA) and were used following isolation for 1 week at this laboratory. After they were weighed, the rats were injected sc with free [^{14}C]MPL or a suspension of liposomes (0.125 mg of [^{14}C]MPL/19.3 μmols of [^{3}H]PC/ml; 0.001 ml/g of body weight) using a Luer tip Hamilton syringe (0.25 ml) fitted with a 30-gauge needle. Five animals were killed at 0.5, 1, 2, 4, 6, 8, and 24 hours after dosing. Each rat was anesthetized with ether, the abdomen was opened, and aortic blood was collected into a heparinized syringe. The skin and subcutaneous tissue at the injection site (about 2 cm in diameter), the right and left inguinal lymph nodes, and a sample of lung tissue were removed and weighed in tared liquid scintillation vials. The tissue samples were digested with Protosol tissue solubilizer (New England Nuclear Corp, Boston, MA) at 50°C overnight. Two milliliters of the resulting solution was mixed with 15 ml of Aquasol scintillation solution (New England Nuclear Corp) and neutralized with 0.10 ml of glacial acetic acid. All samples were equilibrated in a scintillation counter for at least 24 hours prior to counting.

In vivo assay of the effectiveness of liposome-entrapped MPL in the postoperative treatment of lymph node metastasis.—The tumor used was the transplantable mammary adenocarcinoma 13762 which was obtained through the courtesy of Dr. Arthur E. Bogden, Mason Research Institute, Worcester, MA. It was maintained by biweekly trocar transplantation of tissue fragments (about 1–2 mm^3) into the subcutaneous tissue of the flank of female Fischer 344 rats. However, for assay of the chemotherapeutic effectiveness of free or liposome-entrapped MPL, a more uniform rate of tumor growth was essential. This was provided by using tumor cell suspensions which were prepared by sieving fresh tissue taken from peripheral areas of tumor (#60 mesh). Hanks' balanced salt solution (1 ml/g of tissue) was used as the suspending medium. A 0.1-ml aliquot of the suspension was injected sc into the calf of the left leg of rats through a 23-gauge needle. Following randomization of the rats into groups, caliper measurements of the largest and smallest diameters of the tumor were made every 2 days. Ten days after transplantation, radical surgical amputation of the tumor-bearing leg was performed following the method of Karrer and Humphreys (9). To avoid bleeding, the left femoral artery was ligated below the branching of the superficial epigastric artery which supplies blood to the inguinal lymph nodes. After amputation with a guillotine, the wound was examined to ensure removal of the popliteal lymph node, and closed by clamping with metal wound clips. Antibiotic solution (penicillin and streptomycin, 2×10^4 IU and μg/kg, respectively) was injected im into the right thigh. Three days after surgery, a liposome suspension or a solution of free drug was injected near the healing wound. At 22 days after tumor transplantation, all animals were killed; the inguinal lymph nodes were weighed and fixed in 10% Formalin for histologic examination. Two separate chemotherapy experiments were performed, differing only temporally and in the number of treatment groups employed. The doses of liposomal lipid (PC) were 19.6 and 20.9 μmols/kg in experiments 1 and 2, respectively.

RESULTS

Figure 1 shows the elution pattern of aqueous phase MPL-liposomes from a Sephadex G-25M column. Virtually all of the [^{3}H]PC was eluted in a sharp peak, and relatively small proportions of ^{14}C activity and CF, representing entrapped MPL and CF, respectively, were present in these fractions. There was a good separation of free and liposome-entrapped MPL and CF. An interaction of MPL with the lipid phase of liposomes was suggested by differences in the entrapment rates for MPL and CF in these liposomes. The average entrapment rate for CF was 1.4%, whereas for MPL it was 8.1%.

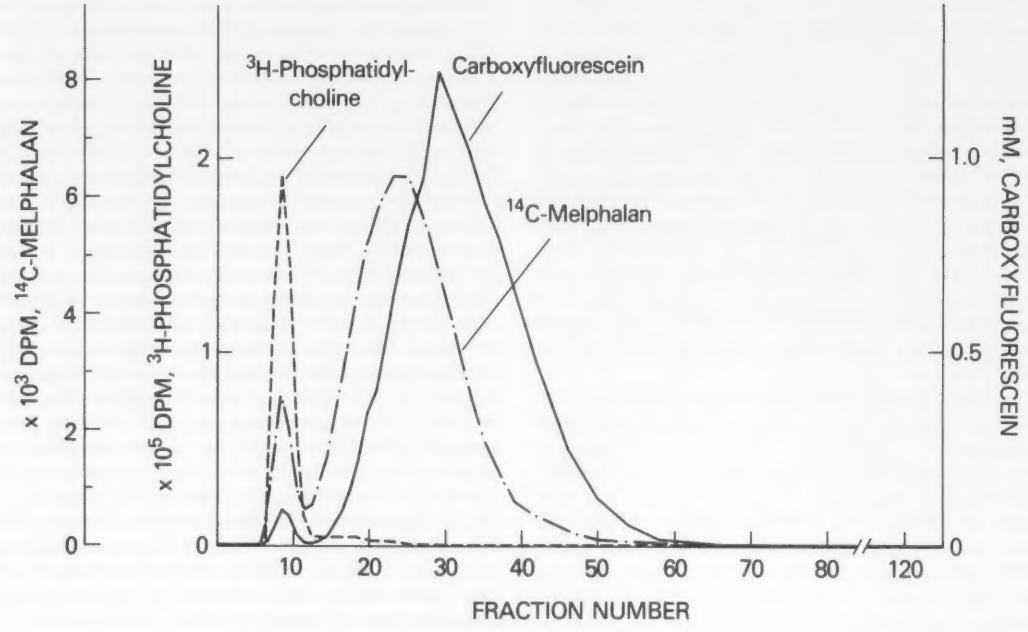

FIGURE 1.—Sephadex G-25M elution profile of liposomes prepared by sonication of lipid film with an aqueous solution containing MPL (1 mg/ml) and CF (20 mM). Concentration of CF in diluted aliquots of each fraction was determined spectrophotofluorometrically.

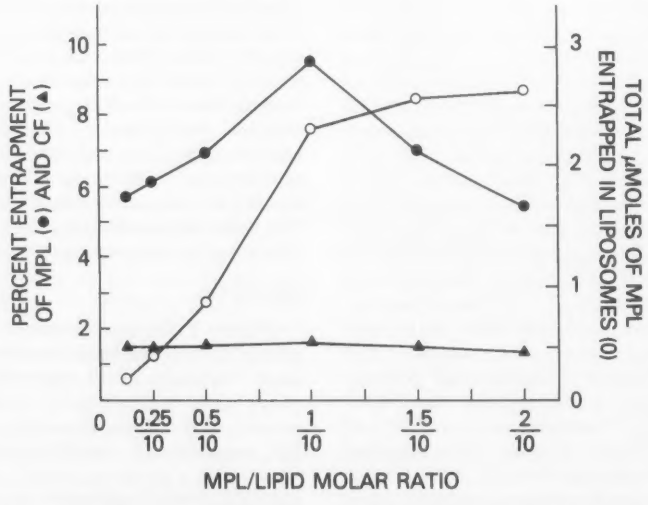

FIGURE 2.—Effect of the MPL/lipid molar ratio on entrapment rate for MPL and CF, and on total μmols of MPL entrapped in liposomes. Liposome suspensions were prepared in which the proportion of lipid used was held constant (240 μmols) while the MPL content was varied from 6 to 48 μmols.

Since it was confirmed in preliminary experiments that CF has little affinity for the liposomal lipids, this difference in entrapment rates suggested that most of the entrapped MPL was associated with the lipid phase of the liposomes. Therefore, it seemed appropriate to prepare lipid phase liposomes in which MPL was codeposited with the liposomal lipid.

The relationship between the MPL/lipid molar ratio and entrapment rate for MPL and CF was next determined by preparing liposome suspensions in which the proportion of lipid used was held constant (240 μmols), while the MPL content was varied from 6 to 48 μmols. The results as shown in figure 2 indicate that the MPL/lipid molar ratio yielding the highest MPL entrapment rate was 1/10, and that further increases in the ratio produced reductions in the MPL entrapment rate. Entrapment of CF was unaffected by the MPL/lipid molar ratio over the ranges tested. In all subsequent experiments, liposomes were prepared at a 1/10 MPL/lipid ratio and the highest MPL concentration obtained was 0.14–0.16 mg/ml of liposome suspension.

The possible decomposition of MPL during the process of liposome preparation was assessed by TLC of authentic MPL, aqueous solutions of MPL subjected to sonication, an MPL solution hydrolyzed by boiling for 3 minutes (13), and liposome-entrapped MPL. Sonication of an aqueous solution of [^{14}C]MPL produced three radioactive zones on TLC, one of which corresponded to authentic MPL (fig 3). Another radioactive zone (R_f = 0.49) became progressively more prominent when sonication was performed at higher temperatures and/or for longer times, and was chromatographically identical to hydrolyzed MPL. On the other hand, the ^{14}C activity entrapped in liposomes was chromatographically identical to authentic MPL. The chromatographic behavior of [^{3}H]PC remained unchanged during sonication under these conditions.

The results of studies on the permeability of liposomes to entrapped MPL and CF are shown in figure 4. At 4°C, both MPL and CF were gradually released from liposomes at almost the same rate (half-life, 5 hours). The release of CF was more rapid at 37°C (half-life, 1.3 hours), and resembled that of MPL at 37°C during the first hour; however, after 4 hours the rate of release of MPL from liposomes was significantly slower than that of CF. At 24 hours, liposomes retained 20% of entrapped MPL, but only 5% of the CF was present.

The results of studies on tissue distribution of free and liposome-entrapped MPL and of liposomal lipid are shown in figure 5 and table 1. Free [^{14}C]MPL was rapidly cleared from the injection site with only 0.6% of the administered radioactivity remaining after 2 hours (fig 5). After sc injection of [^{14}C]MPL/[^{3}H]PC-liposomes, the rate of clearance of tritium was considerably slower than that of ^{14}C activity. Nevertheless, about 4% of ^{14}C activity was still present at the injection site at 2 hours after treatment. After injection of free [^{14}C]MPL, the concentration of MPL equivalents in ipsilateral lymph nodes showed a transient increase over those in contralateral nodes at 0.5 hour, but by 1 hour the levels in ipsilateral and contralateral inguinal nodes were similar (table 1). The concentrations of MPL equivalents in plasma and lung were comparable and showed a slow and steady decrease throughout the course of the 24-hour experiments. The differential in MPL equivalent concentrations between ipsilateral nodes and plasma ranged between 2 and 3 up to 24 hours after dosing. In contrast, the ipsilateral lymph node concentration of MPL equivalents was fivefold higher than the plasma concentration from 0.5 to 2 hours after dosing with MPL-liposomes, and after 2 hours it ranged between 10 and 20 times higher than the plasma concentration. The concentrations of MPL equivalents in plasma, lung, and contralateral nodes were comparable, and the clearance of MPL equivalents from contralateral lymph nodes, plasma, and lung of rats receiving MPL-liposomes closely resembled the pattern noted in rats treated with free MPL. Liposomal entrapment produced a significant and sustained increase in lymph node uptake of MPL. The concentration of PC equivalents in ipsilateral lymph nodes markedly increased with time after dosing. It exceeded that in plasma by ≥ 80-fold at all time intervals tested, and at 24 hours after dosing it was 200 times higher than that in plasma.

The results of two separate chemotherapy experiments are summarized in table 2. At the time of surgery, the size of the primary tumor averaged 153 ± 20 mm^2 in experiment 1 and 210 ± 38 mm^2 in experiment 2, and it was readily distinguished from normal tissue. The weight of the inguinal lymph node on the day of treatment was 120 ± 16 mg. There was no significant decrease in body weight following postoperative MPL chemotherapy with the range of doses used. When surgery was followed by treatment with free MPL, the reduction in weight of lymph nodes correlated with the drug dose. In both experiments, a greater reduction in lymph node weight was produced by liposome-entrapped MPL (0.125 mg/kg) than by the same dose of free MPL alone or mixed with control liposomes. Histologic examination of lymph nodes from control and treated animals on Day 22 revealed that enlarged inguinal lymph nodes (> 80–90 mg) were almost entirely occupied by metastatic tumor; inguinal nodes weighing < 80 mg usually contained micrometastases located in the subcapsular region. Although metastases

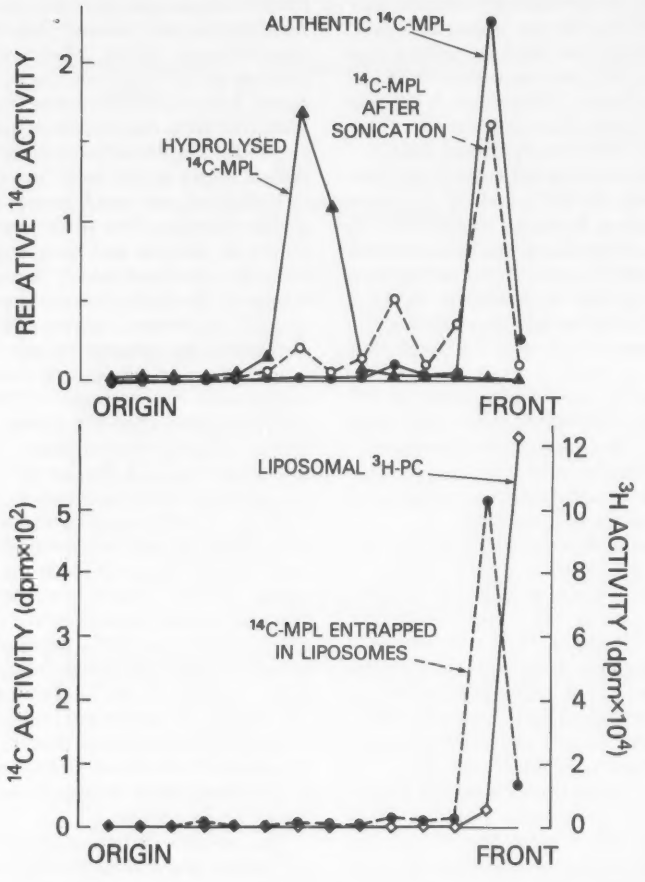

FIGURE 3.—TLC of authentic [^{14}C]MPL and [^{14}C]MPL after sonication, hydrolysis, or entrapment in liposomes. Hydrolysis of MPL was achieved by boiling an aqueous MPL solution for 3 mins.

were observed histologically in lumbar lymph nodes, most of the nodes were not enlarged on Day 22.

DISCUSSION

Our studies on the disposition of MPL following sc injection demonstrated clearly that liposomal entrapment retards the absorption of drug from the injection site and enhances its uptake by regional lymph nodes. Free MPL injected sc appeared to be absorbed primarily via blood capillaries; however, there was evidence of some direct absorption into regional lymph nodes via lymphatics shortly after injection which might have been due to local injury and/or increased interstitial pressure (1,6). A similar but larger transient peak of MPL equivalents in ipsilateral nodes was also noted after injection of MPL-liposomes which might be partly due to a pulse of free MPL released from liposomes shortly before and/or after injection. While the concentration of MPL equivalents in lymph nodes, plasma, and lung fell gradually with time after dosing with free MPL, the ipsilateral lymph nodes of rats receiving liposome-entrapped MPL showed a steady increase in the concentration of MPL equivalents. This accumulation began at 2 hours after dosing and contin-

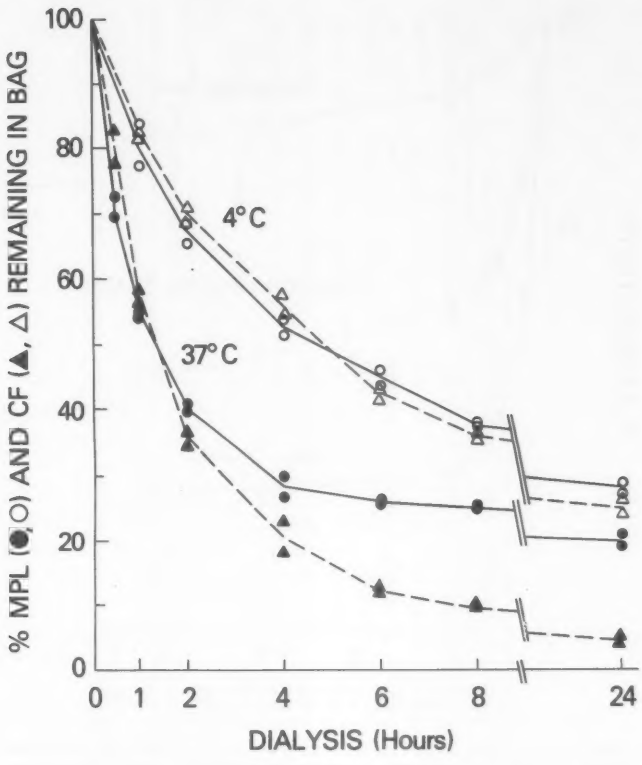

FIGURE 4.—Release of MPL and CF from liposomes incubated at 4°C and 37°C. Liposomes in which [^{14}C]MPL and CF were entrapped were dialyzed in cellulose bags against 150 volumes of PBS; proportion of radioactivity and fluorescence remaining in the bag was determined at intervals.

ued to at least 24 hours. The accumulation of MPL equivalents in ipsilateral lymph nodes was paralleled by a corresponding accumulation of PC equivalents.

These results indicate that MPL-liposomes injected sc provide a depot from which radioactivity is gradually absorbed. Whether the accumulation of both tritium and ^{14}C activity in ipsilateral nodes represents uptake of intact MPL-liposomes cannot be determined from our results, nor is it possible to assume that the radioactive material in nodes was carried from the injection site by lymphatic channels. However, the observation that from 4 to 24 hours after dosing with MPL-liposomes levels of ^{14}C in ipsilateral lymph nodes were strikingly proportional to tritium levels suggests that MPL was absorbed via lymphatics in association with liposomal lipid. Following sc injection, MPL-liposomes could reach regional lymph nodes by passive diffusion through the interstitial space or by absorption into blood and/or lymph capillaries. However, the concentration of both MPL and PC equivalents in plasma, as well as in contralateral nodes and lung, was significantly and consistently lower than that in ipsilateral nodes throughout the 24-hour experiments. This finding suggests that blood capillaries are not the major route by which MPL-liposomes are carried from the interstitial space to regional nodes. Furthermore, it is unlikely that passive diffusion could account for a significant proportion of the radioactivity reaching the regional lymph node, since it has been shown that liposomes do not readily diffuse through the interstitial space (15,16,19). The use of small liposomes in this study was based on

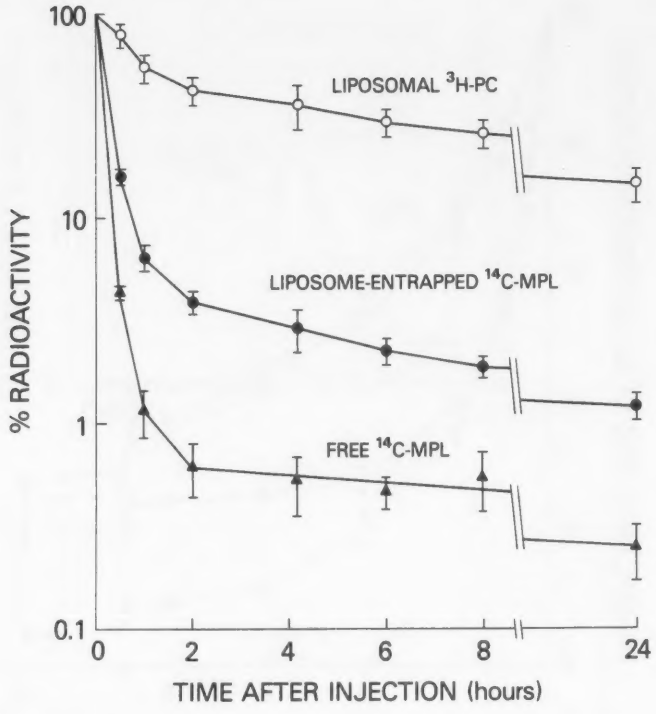

FIGURE 5.—Clearance of free and liposome-entrapped [^{14}C]MPL and liposomal [^{3}H]PC from the subcutaneous injection site. 5 animals for each group were injected sc with free [^{14}C]MPL or a [^{14}C]MPL/[^{3}H]PC-liposome suspension (0.125 mg of MPL/ml, 0.001 ml/g of body weight).

earlier work which indicated that vesicle size may be an important factor in the uptake of liposomal lipid and entrapped agent by regional lymph nodes following interstitial injection. Thus, Segal et al (22) administered liposomes containing dactinomycin to rats by intratesticular injection, and reported a higher uptake of drug and liposomal lipid when sonicated small liposomes were administered than when large multilamellar vesicles were employed. Similar observations were made by Ryman et al (21) and Richardson et al (20) in their studies on the lymph node uptake of neutral and positively charged liposomes from the interstitial space.

Our studies with the mammary adenocarcinoma 13762 have shown that following surgical resection of the primary sc tumor, postoperative sc treatment with free MPL produced a dose-related decrease in the growth of lymph node metastases. Entrapment of MPL in liposomes produced a significant increase in its effectiveness against the growth of lymph node metastases. Thus, when entrapped in liposomes, MPL at a dose of 0.125 mg/kg produced a significant reduction (47%–50% of controls treated by surgery alone) in the weight of lymph node metastases; to obtain an equivalent effect against metastatic growth in lymph nodes with free MPL, it was necessary to administer an MPL dose four to eight times higher (0.5–1.0 mg/kg). The increase in effectiveness against lymph node metastasis was not observed when free MPL was mixed with control liposomes immediately prior to dosing. Therefore, MPL must actually be entrapped within liposomes to enhance its effects on tumor growth in regional nodes.

How liposomes and entrapped drug interact with tumor cells is not clear. Most studies on the interaction of liposomes with cells have employed in vitro conditions. The results of the dialysis experiments showed that both CF and MPL were released from

TABLE 1.—Concentration of MPL and PC equivalents in lymph nodes, plasma, and lung of rats treated with free [^{14}C]MPL or with [^{14}C]MPL/[^{3}H]PC-liposomes*

Time after dosing (hrs)	MPL equivalent concentration (ng/g or ml) after dosing with free MPL				MPL equivalent concentration (ng/g or ml) after dosing with MPL-liposomes				PC equivalent concentration (nmols/g or ml) after dosing with MPL-liposomes			
	Lymph nodes				Lymph nodes				Lymph nodes			
	Left	Right	Plasma	Lung	Left	Right	Plasma	Lung	Left	Right	Plasma	Lung
0.5	447[a] (17)	169 (14)	122 (15)	134 (12)	572[a] (46)	98.4 (16.5)	115 (11)	112 (10)	318 (186)	0.74 (1.09)	2.59 (1.12)	4.15 (1.85)
1	176[a] (36)	136 (36)	73.1 (6.2)	98.6 (19.2)	406[a] (112)	75.8 (13.8)	77.4 (8.2)	62.0 (17.0)	390 (132)	1.64 (0.81)	5.01 (1.94)	4.20 (1.71)
2	158[b] (46)	105 (22)	51.9 (3.1)	71.2 (10.1)	267[b] (63)	65.2 (43.1)	58.6 (5.8)	50.7 (12.1)	346 (73)	2.12 (0.87)	5.11 (0.57)	3.57 (0.45)
4	116[c] (8)	84.2 (15.7)	38.8 (3.8)	55.0 (9.0)	358[c] (69)	37.9 (13.8)	41.2 (2.1)	30.7 (3.4)	637 (165)	2.27 (0.57)	7.99 (0.99)	5.46 (0.92)
6	86.0[c] (24.1)	59.4 (13.1)	32.9 (3.4)	37.2 (10.3)	306[c] (59)	45.4 (21.1)	31.7 (5.5)	34.8 (20.6)	685 (200)	5.34 (0.80)	7.88 (1.04)	8.15 (0.75)
8	80.2[c] (15.2)	47.2 (12.6)	31.1 (3.3)	34.6 (5.7)	346[c] (39)	28.4 (21.6)	32.5 (1.9)	31.1 (13.4)	869 (134)	6.66 (2.49)	7.18 (0.74)	8.80 (0.67)
24	65.6[c] (10.7)	48.2 (15.5)	24.0 (1.3)	30.0 (7.8)	539[c] (80)	40.3 (16.1)	22.6 (2.2)	23.2 (4.2)	1359 (269)	12.23 (2.33)	6.63 (0.87)	12.19 (1.28)

*Concentration derived from ^{14}C or ^{3}H activities of tissues. Values = mean of ≥ 4 animals. Nos. in parentheses = SD. A significant difference between corresponding values for free and liposome-entrapped MPL was noted in regional (left) lymph nodes: (*a*) $P < 0.01$, (*b*) $P < 0.05$, and (*c*) $P < 0.001$.

TABLE 2.—Effect of postoperative chemotherapy with free or liposome-entrapped MPL on lymph node metastases of mammary adenocarcinoma 13762*

Treatment	Inguinal lymph node weight (g)†	
	Experiment 1	Experiment 2
None	0.70 ± 0.14 (13)	0.64 ± 0.11 (11)
Free MPL (1 mg/kg)	0.30 ± 0.08 (12)	0.27 ± 0.06 (10)
Free MPL (0.5 mg/kg)	0.37 ± 0.11 (12)	nd
Free MPL (0.25 mg/kg)	0.50 ± 0.10 (11)	nd
Free MPL (0.125 mg/kg)	0.51 ± 0.13 (14)	0.62 ± 0.10 (11)[a]
Control liposomes	0.68 ± 0.10 (13)	nd
Free MPL (0.125 mg/kg) plus control liposomes	0.57 ± 0.12 (14)	0.51 ± 0.08 (12)[b]
Liposome-entrapped MPL (0.125 mg/kg)	0.33 ± 0.07 (14)	0.32 ± 0.05 (12)[a,b]

*Rats were inoculated in the calf sc with a tumor cell suspension, and the tumor-bearing leg was amputated 10 days later. 3 days after surgery, rats were given a single sc injection of free MPL, control liposomes, free MPL mixed with control liposomes, or liposome-entrapped MPL. Animals were killed 22 days after tumor inoculation and the weight of ipsilateral inguinal lymph nodes was recorded.

†Mean ± SE; values in parentheses = No. of rats in each group. The difference was significant with (a) $P < 0.02$ and (b) $P < 0.05$; nd = not determined.

liposomes at a relatively high rate during the first 4 hours of dialysis, raising the possibility that MPL exerts a destabilizing effect on the liposome membrane. Nevertheless, 20% of MPL remained entrapped within liposomes at the end of the 24-hour dialysis period. As suggested by the results of dialysis experiments, liposomes are likely to release entrapped drug in the regional nodes as well as at the injection site at least during the initial phase. This local release could allow for a continuous and concentrative uptake of MPL by target cells in the tissue (24).

Fisher et al (7) have reported results of a study conducted at 37 hospitals using MPL (0.15 mg/kg/day for 5 days every 6 weeks) in patients having positive nodes after mastectomy. The data at 2 years indicated that the recurrence rate was significantly reduced in patients who received chemotherapy (8). The rationale of employing chemotherapy as an adjuvant to surgery is based on the concept that tumor cells are disseminated at the time of surgery and metastases can be prevented more effectively by early chemotherapy.

The mammary adenocarcinoma 13762 in the Fischer 344 rat is a well-studied transplantable syngeneic mammary tumor system of potential use in evaluating the antimetastatic effects of chemotherapeutic agents. Bogden et al (2–4) have treated this tumor with single agents with clinical activity as well as with combined modality regimens. Their studies show that this tumor is very responsive to MPL. Bogden et al (2,3) and Kreider et al (10,11) described the occurrence of lymph node and lung metastases following local excision of this tumor implanted sc. It has not yet been possible to establish a procedure for producing lymph node metastases exclusively; surgical removal of the primary tumor earlier than 5 days after inoculation reduces the incidence of lung metastases but also renders the development of lymph node metastases less reproducible. Therefore, although MPL-liposomes were more effective against lymph node metastases than free MPL, survival might not be prolonged because of death from lung metastases. To confirm the effectiveness of liposome-entrapped drug therapy in prolonging survival, it will be necessary to establish a model of lymph node metastasis in which the primary tumor is removed prior to the development of lung metastases. It will also be necessary to examine factors influencing lymphatic absorption of liposomes and to evaluate the relationship between those physical characteristics of liposomes (eg, lipid composition) which may affect their stability, permeability, and in vivo effectiveness. Such studies are currently underway in this laboratory.

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Serial Analysis of Melanoma Growth Kinetics in a Patient Receiving Bleomycin and Procarbazine Therapy: Comparison of Tumor Volume, Flow Cytometry, and Thymidine Labeling Techniques [1,2]

James J. Stragand, Benjamin Drewinko,* Barthel Barlogie, Nicholas Papadopoulous, and Robert A. White [3,4]

SUMMARY

A serial growth-kinetics analysis of the tumor of a patient with melanoma who was receiving bleomycin and procarbazine demonstrated significant reduction in cell proliferation. This reduction was followed by prompt proliferative recovery, which involved a parasynchronous movement of cells from $G_1 \rightarrow$ DNA $\rightarrow$ mitosis. However, no change in the mean tumor volume of 35 cutaneous lesions was observed during or after treatment. These results suggest that tumor volume measurements alone do not provide a complete evaluation of chemotherapeutic response and/or drug efficacy.

[Cancer Treat Rep 66:529–534, 1982]

Malignant melanoma is an especially difficult neoplasm to treat because it has an unpredictable growth pattern and is refractory to most chemotherapeutic strategies. Of the many agents tested, DTIC has exhibited the best results, although the overall response is only 20%. Other agents such as procarbazine have shown a response of approximately 28% in limited clinical studies (1). No combination of agents has been shown to produce a better overall response than DTIC alone, although a recent report indicates a 40% response rate in patients receiving lomustine (CCNU), vincristine, and bleomycin after failure to respond to DTIC therapy (2).

In these cases and typically for human tumors, therapeutic efficacy is evaluated in terms of tumor volume reduction. However, tumor regression is subject to many variables, including the cell loss rate, local edema, and the elimination of nontumorous elements such as collagen (3). Therefore, regression may reflect individual tumor characteristics rather than therapeutic effects.

In an effort to evaluate the response of melanoma lesions in terms of both tumor volume and cytokinetics before and during systemic chemotherapy, we have performed a serial cell kinetic and tumor volume analysis on a patient undergoing bleomycin and procarbazine treatment.

MATERIALS AND METHODS

Patient Treatment and Tumor Preparation

The patient examined in this study was a 58-year-old white female initially diagnosed as having melanoma in 1972 when a mole on her mid-upper back was removed. The same area was re-excised in 1979, together with a nodule from the left flank. Following the second surgical procedure, the patient received three courses of chemotherapy consisting of vincristine, dactinomycin, DTIC, and hydroxyurea

[1]Received May 22, 1981; revised Aug 21, 1981; accepted Sept 18, 1981.

[2]Supported by Public Health Service grants CA-14528 and CA-11520 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Departments of Laboratory Medicine (*J. J. Stragand* and *B. Drewinko*), Developmental Therapeutics (*B. Barlogie*), Medicine (*N. Papadopoulous*), and Biomathematics (*R. A. White*), The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

[4]We acknowledge the skilled technical assistance of Ms. Cris Sampson and Ms. Shields Sloan.

**Reprint requests to:* Dr. Benjamin Drewinko, Section of Hematology, Department of Laboratory Medicine, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

over a 3-month period. During this treatment, several new cutaneous lesions appeared on the thighs, upper arms, and thorax. An additional course of chemotherapy was attempted, using high-dose DTIC given as a bolus. This therapy produced severe myelotoxicity and was discontinued. Immunotherapy using BCG and *Corynebacterium parvum* was ineffective.

A course of chemotherapy consisting of bleomycin (5 mg iv on Days 1–7) plus procarbazine (50 mg orally on Day 1, 100 mg orally on Day 2, and 150 mg orally on Days 3–14) was chosen. Prior to the start of therapy, the patient had approximately 75 palpable cutaneous lesions ranging in size from 0.02 to 36.5 cm^3. Of these lesions, 30 had well-defined margins and could be accurately and reproducibly measured with calipers. Throughout the treatment period, these lesions were monitored by biweekly caliper measurements in two dimensions, and the volume was calculated as: $V = \pi/6 \quad [\text{length} \times (\text{width})^2]$.

Since the patient expressed the desire to have several lesions removed from the head and upper chest for cosmetic reasons, it was decided to remove two or three lesions at intervals to coincide with the start, midpoint, and completion of therapy. Within 15 minutes of removal, the lesions were minced with scissors in warm saline and filtered through a stainless steel grid. The resulting monocellular suspension was employed in the following cell kinetics analyses.

Autoradiographic Analyses

Primer-available α-DNA polymerase (PDP) assay.—This assay is an autoradiographic method for the detection of nuclear α-DNA polymerase within a cell sample (4). α-DNA polymerase has been demonstrated in actively cycling cells, regardless of the particular cell cycle phase. Quiescent (G_0) or dead cells lack this enzyme marker. The cell sample was placed on acid-cleaned glass slides and air-dried, and the cytoplasm was removed by dipping in 0.25% agar at 42°C. A glass ring was then placed around the sample with wax, and the nuclei were incubated with all the precursors necessary for the synthesis of DNA, including tritiated thymidine triphosphate (^{3}H-TTP), but *without* α-DNA polymerase or DNA template. If the sample nuclei contain α-polymerase plus template, DNA synthesis occurs, and the cells can be identified by autoradiographic detection of incorporated ^{3}H-TTP. The PDP index (the number of cells incorporating ^{3}H-TTP over the total number of cells examined) provides a reliable estimate of the number of actively cycling cells within the tumor (tumor growth fraction) (5,6).

Tritiated thymidine (^{3}H-Thd) labeling index (LI).—The ^{3}H-Thd LI represents the fraction of cells that are synthesizing DNA at the time ^{3}H-Thd is added. The incorporation of ^{3}H-Thd can be quantified autoradiographically, and the resultant LI provides an estimate of the number of DNA-synthesizing, or S-phase, cells in the tumor population. The cell suspension was resuspended in 5 ml of RPMI-1640 medium plus 10% fetal calf serum, to which 2.5 microcuries/ml of ^{3}H-Thd had been added. The reaction was terminated on ice 30 minutes later; the cells were washed and slides were prepared for autoradiographic analysis. The number of mitotic figures was also determined in the ^{3}H-Thd incorporation studies.

All autoradiographic analyses employed NTB 2 emulsion (Eastman Kodak, Rochester, MN) in a 1:1 dilution with triple-distilled water and slides that were exposed for 3–5 days for LI examinations or for 14 days for PDP determinations. Developing employed a gold-activated procedure to enhance the latent image and reduce exposure times (7). In all studies, pilot slides were developed every other day. Grain counts and percent labeled cells increased gradually until only the number of grains per cell increased but the percent labeled cells plateaued. One thousand cells were examined for both the PDP and ^{3}H-Thd indices.

Flow Cytometric Analysis (FCM)

This procedure allows the determination of DNA content within individual cells, which can then be used to infer their position within the cell cycle; ie, a 2N content would be indicative of a cell in G_1 or G_0, whereas a 4N content would be indicative of a cell within the G_2 or mitotic compartment. The cell suspension was fixed in 70% ethanol, treated with 0.5% pepsin at 27°C for 5 minutes, buffered, and then stained with ethidium bromide and mithramycin. Cells were analyzed using a Phywe ICP 11 flow cytometer. DNA-compartment distributions were analyzed by the method of Johnston et al (8), employing a constrained Gaussian program to determine fractions of cells within the various DNA compartments.

RESULTS

Tumor Growth

Tumor growth during both the treatment and recovery periods indicated no significant changes in the mean volume of the lesions examined. Individual lesions exhibited some fluctuations, both increasing and decreasing in size. However, no significant change in the mean tumor volume could be estab-

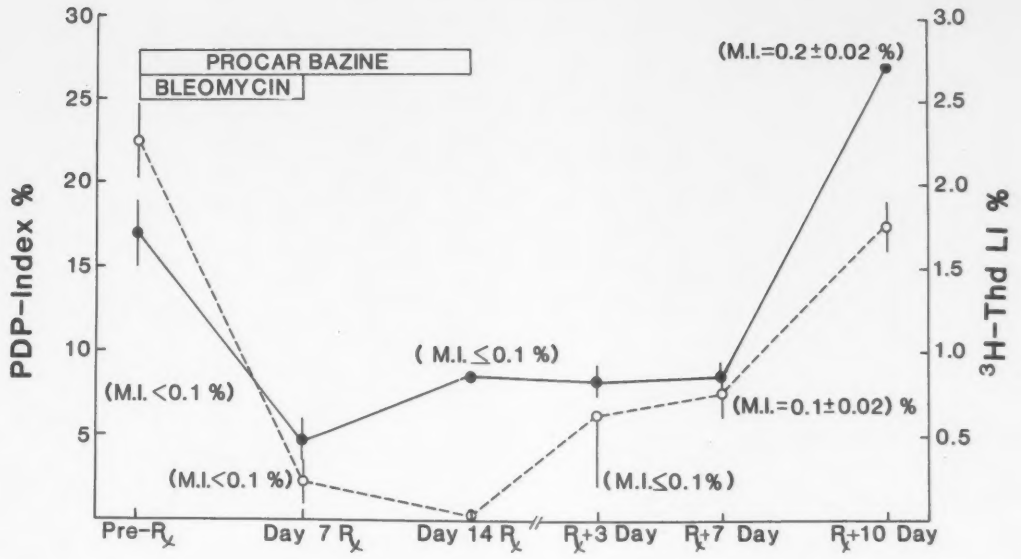

FIGURE 1.—Response in PDP (●——●) and ^{3}H-Thd LI (○——○) to bleomycin and procarbazine therapy. Mitotic indices (MI) are indicated adjacent to data points. Mean of 2–3 lesions per point ± 1 SE.

lished whether the tumors were grouped according to size, position on the body, or time of appearance.

Tumor Cell Kinetics

The cell kinetic effects of the bleomycin and procarbazine therapy are presented in figure 1. The pretreatment cell kinetic profiles indicated a tumor growth fraction of 17.0% ± 3.0% and a ^{3}H-Thd LI of 2.25% ± 0.51%. Of the 1000 cells examined for the ^{3}H-Thd LI, no mitotic figures were observed, indicating a mitotic index < 0.1%. The population consisted of > 95% mononuclear cells, of which approximately 30% showed significant melanin concentration.

On Day 7 of therapy, the PDP index was reduced by a factor of 3, to 4.9% ± 0.2%, whereas the ^{3}H-Thd LI was reduced by a factor of 10, to 0.22% ± 0.1%. On Day 14 of therapy, the PDP index exhibited a slight increase to 8.5% ± 0.1%, whereas the ^{3}H-Thd LI was reduced to zero, ie, no demonstrable cells incorporating ^{3}H-Thd. The mitotic index during these treatment intervals showed no change from the pretreatment level (< 0.1%).

Between Days 3 and 10 after the cessation of therapy, a series of cell kinetic events were observed. On Day 3, there was an elevation in the ^{3}H-Thd LI, with no significant changes in either the PDP or mitotic indices. On Day 7 after therapy, there was an elevation of the mitotic index to 0.1% ± 0.02%, with no further increases in the PDP or the ^{3}H-Thd LI. On Day 10 after therapy, elevations of both the PDP and the ^{3}H-Thd LI were observed, with a further increase in the mitotic index to 0.2% ± 0.2%.

FCM analyses before, during, and after bleomycin and procarbazine treatment are presented in figure 2. Results of computer analyses of these distributions to determine relative proportions of cells within the various cell cycle phases are given in table 1. Granulocyte standards from normal volunteers indicated that pretreatment melanoma samples did not exhibit DNA content abnormalities. There was a close agreement between the pretreatment ^{3}H-Thd LI (2.2% ± 0.5%) and the S fraction obtained by the FCM technique (3.3%). The absence of cytologically demonstrable mitotic figures indicated that the G_2 + M (5.0%) fraction was comprised predominantly of G_2 cells. The skewed appearance of the G_2 + M peak may reflect the small proportion of binucleate cells (< 5%) demonstrable in cytologic preparations or a small fraction of cells with tetraploid DNA content.

On Day 7 of the bleomycin and procarbazine ther-

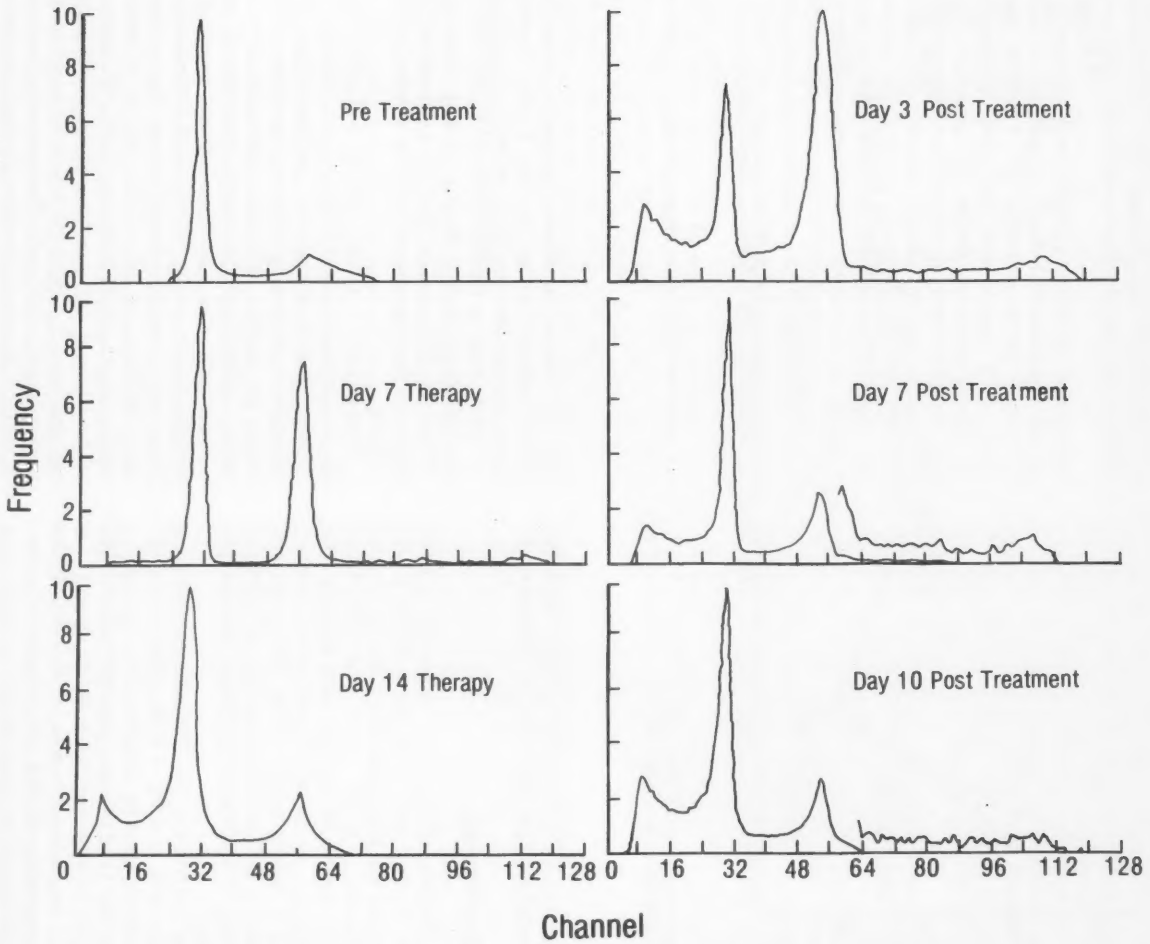

FIGURE 2.—Composite DNA histograms for 2–3 lesions per sampling period, obtained before, during, and after bleomycin and procarbazine therapy. Mean coefficient of variation was 4.5% ± 0.3% (range, 2.1%–6.9%).

TABLE 1.—FCM cell cycle distributions before, during, and after bleomycin and procarbazine treatment

Sample	% in phase—		
	G_1	S	G_2 + M
Before treatment	91.6	3.3	5.0
During treatment			
Day 7	40.5	1.5	58.0
Day 14	37.5	26.4	36.1
After treatment			
Day 3	21.4	10.6	68.0
Day 7	5.0	60.6	34.4
Day 10	56.7	15.4	27.9

apy, a tenfold increase (58%) of the peak at channel 56 was observed. This peak could represent an accumulation of diploid cells in G_2 phase, the appearance or enhancement of a tetraploid $G_{1/0}$ population, or both. On Day 14 of therapy, the peak at channel 56 was slightly reduced (36%). This decrease may reflect individual variations within the melanoma lesions or may involve the elimination of G_2-blocked cells. Supporting this latter assumption is the appearance, for the first time, of cell debris (far-left channels).

On Day 3 after treatment, the accumulation of cells at channel 56 was enhanced slightly (68%) and a new peak appeared at channel 112. These events suggest the endomitotic replication of a portion of the G_2-blocked cells and the distribution of a tetraploid population in the various compartments of the cell cycle. The peaks in channels 56 and 112 were reduced by Day 7 after treatment, with the latter peak disappearing by Day 10.

Significant fluctuations in the S fraction occurred throughout all sampling periods, but these changes did not correlate with the ^{3}H-Thd LI measurements on the same sample.

DISCUSSION

The results of the present study indicate that bleomycin and procarbazine therapy had a minimal effect on the measured melanoma tumor volume. Although tumor size is often used as an indicator of chemotherapeutic effectiveness, this parameter may be misleading. The volume response of the tumor following treatment with cytotoxic agents is a combination of several factors, including suspension of cell renewal, inherent cell loss rate, edema, necrosis, and the elimination of noncellular components. In the present study, although no changes were observed in tumor volume, a marked reduction in proliferative capacity was observed during the 14-day treatment period, with a marked proliferative recovery seen between Days 3 and 10 after treatment.

The initial cell kinetic profile of this patient indicated a low proliferative profile for the melanoma lesions: ie, a tumor growth fraction of approximately 15% and a ^{3}H-Thd LI of approximately 2%. These kinetic values agree well with the proliferative profile of the amelanotic subgroup reported by Newburger and Weinstein (9). The low proportion of cells that would be sensitive to treatment with cycle-active agents probably contributed to the lack of an observed tumor volume response. The cell kinetic response indicated that the DNA-synthesizing cells were especially sensitive to this therapy and were reduced to 0 after 14 days. However, after treatment, a significant population of PDP-positive (actively cycling) cells remained. These cells represent either resistant cells in the G_1 or G_2 periods or cells recruited into cycle to replace cells killed during earlier periods.

One explanation for these cytokinetic events is based on the presence of a resistant population of cells with a G_1 DNA content. On recovery from drug treatment, these cells progress through the cycle, producing the increase in the ^{3}H-Thd LI that occurs on Day 3 with the start of DNA synthesis and producing the subsequent mitotic response observed between Days 7 and 10. The sequential appearance of DNA-synthesizing cells followed by the appearance of mitotic cells is typical of a parasynchronous movement of G_1-blocked cells through the cell cycle. Following the doubling of this cell cohort at mitosis, the population progresses through the cycle, producing the elevation of both the PDP index and the ^{3}H-Thd LI on Day 10.

An alternative hypothesis involves the recruitment of cells from G_0 and/or the release of viable cells from a G_2 cycle block. Indeed, the kinetics of tumor recovery may represent a combination of the above possibilities. FCM methodology provided additional cell kinetic details underlying the response of the melanoma lesions to bleomycin and procarbazine. On Day 7 of therapy, a marked accumulation of cells at channels typical for G_2 + M cells was consistent with the reported action of bleomycin as a G_2 arrest agent (10) or with the appearance of a tetraploid $G_{1/0}$ population resulting from endomitotic replication. In either case the magnitude of this increase (58%) implies the sequestering of the original cycling population (15% as measured by the PDP assay) and the accumulation of cells recruited from G_0. We conclude from the present study that tumor volume measurements alone do not provide an accurate estimation for chemotherapeutic effectiveness since major cytotoxic and cytokinetic

changes may not be reflected by decreased tumor size over a restricted time period. Thus, active drugs with potential benefits for the patient may be discarded when only this parameter is used to assess the efficacy of an antitumor regimen. Therefore, additional measurements such as those described in this report may be significant in avoiding false-negative conclusions regarding an anticancer agent.

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Effect of Cytarabine on Cytotoxic T-Cell Development[1,2]

Vincent J. Merluzzi,* Brian M. Susskind, and Ronald B. Faanes[3]

SUMMARY

Administration of repeated high doses of cytarabine to normal C57BL/6 mice causes a depression of in vitro cytotoxic T-cell development against allogeneic tumor cells. This low cytotoxic T-cell response occurs in the absence of a reduction in cytotoxic T-cell precursor cells. The low cytotoxic response can be restored to normal levels when a source of helper factor is added to the culture system.

[Cancer Treat Rep 66:535–540, 1982]

Cytarabine (ara C), an inhibitor of DNA synthesis (1,2), has been used effectively in the treatment of experimental tumors in mice (3–7) and is an essential component of successful therapy programs for the treatment of patients with acute leukemia (8–16). Early studies in mice have shown that the maintenance of relatively constant levels of ara C in body fluids by continuous infusion is a more effective approach to leukemic cell destruction than single high-dose therapy (6).

Recent studies of patients with acute leukemia have shown that repeated iv infusion of ara C induces remission in 11 of 14 patients (12). Another study has shown that leukemic patients refractory to conventional chemotherapy respond to high-dose (1.0–7.5 g/m^2) ara C therapy (13).

As with most toxic compounds, a compromise must be achieved whereby high blood levels of the drug or its active metabolites are maintained for tumor cell destruction, with some degree of selective toxicity to the neoplastic cells relative to normal tissue. Alternatively, a rescue regimen for normal tissue may be initiated.

The purpose of this study was to analyze the effects of repeated high-dose therapy with ara C on the immune responsiveness of normal non-tumor-bearing mice. The experiments in this study focus on the ability to "rescue" or protect the cytotoxic T-cell (CTL) arm of the immune response.

METHODS

Mice and Cell Lines

Male C57BL/6 (H-2^b) and DBA/2 (H-2^d) mice (6–8 weeks old) were obtained from The Jackson Laboratory, Bar Harbor, ME.

P815 (H-2^d) mastocytoma cells and EL4 (H-2^b) lymphoma cells were maintained by weekly ip passage in DBA/2 and C57BL/6 mice, respectively. C118 (H-2^k) myeloma cells were maintained by in vitro cultivation in RPMI medium (Grand Island Biological Co, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum.

Ara C

Ara C was obtained from the Cancer Chemotherapy National Service Center, Bethesda, MD, and from Dr. Franz Schmid, Sloan-Kettering Institute. The compound was dissolved in sterile saline at a concentration of 4 mg/ml and injected ip into individually weighed C57BL/6 mice once a day for 6 consecutive days (30–120 mg/kg). Control mice received an equivalent volume of sterile saline. The LD10 for this schedule has been previously determined to be 60 mg/kg (17). Two to four mice were used per experimental variable.

[1]Received Aug 14, 1981; revised Nov 2, 1981; accepted Nov 9, 1981.

[2]Supported in part by Public Health Service grants CA-26335, CA-25608, and CA-16271 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Sloan-Kettering Institute for Cancer Research, Donald S. Walker Laboratory, Rye, NY.

*Reprint requests to: Dr. Vincent J. Merluzzi, Sloan-Kettering Institute for Cancer Research, Donald S. Walker Laboratory, 145 Boston Post Rd, Rye, NY 10580.

Preparation of Spleen Cells

Spleen cells were prepared as described previously (18). Briefly, spleens were aseptically removed and teased into single-cell suspensions in balanced salt solution, large debris were allowed to settle out, and the cells were washed three times in balanced salt solution.

In Vitro Generation of CTL

Splenic lymphocytes were cultured at 5×10^6/ml with 2.5×10^5 mitomycin-treated (10^7 cells/ml plus 50 μg/ml of mitomycin for 30 mins at 37°C) P815 tumor cells/ml in a final volume of 0.2 ml. Cultures were grown in flat-bottom microculture plates (No. 76-003-05; Linbro Scientific, Hamden, CT) for 5 days at 37°C in air containing 5% CO_2. Two to four spleens were pooled and used for each experimental group and 12–24 cultures were used per experimental variable.

CTL Assay

The CTL response was determined on the fifth day of incubation by a modification of the standard ^{51}Cr release assay (19). Briefly, $2–4 \times 10^6$ P815 tumor cells were mixed with 200 microcuries of $Na_2{}^{51}CrO_4$ in 0.5 ml of RPMI containing 10% calf serum (CS) and incubated at 37°C for 1 hr. The ^{51}Cr-labeled cells were washed three times by centrifugation in RPMI containing 20% CS, followed by suspension in RPMI containing 10% CS.

The cultured splenocytes were harvested, washed, counted, suspended in RPMI with 10% CS, and plated in 0.1-ml aliquots in conical well microculture plates (Linbro Scientific). A 100-μl aliquot of P815 tumor cell suspension, containing 5×10^3 ^{51}Cr-labeled target cells, was added per well. The lymphocyte:target cell ratios were generally 50:1, 25:1, 12:1, and 6:1, set up in quadruplicate cultures. The plates were centrifuged at $30 \times g$ for 3 mins and incubated for 5 hrs at 37°C in air with 5% CO_2. After the 5-hr incubation, the plates were centrifuged at $400 \times g$ for 5 mins and the radioactivity released was determined by analyzing 0.1 ml of each culture well supernatant in a Nuclear Chicago gamma counter. Percent specific ^{51}Cr release was calculated as follows:

$$\frac{\text{experimental release–spontaneous release}}{\text{total releasable counts–spontaneous release}} \times 100$$

Total releasable counts were determined from a distilled water lysate of the target cells and were always ≥ 85% of the total input. The spontaneous release values were determined from cultures containing target cells alone and were always < 15%.

When cytotoxic activity was measured at several lymphocyte:target cell ratios, the data were further analyzed as described (19). Cytotoxic activity varies as a function of the logarithm of the CTL:target cell ratio. The data, therefore, were plotted and the lymphocyte:target cell ratio necessary to obtain 33% lysis was extrapolated from the linear portion of the curve. This number of lymphocytes was arbitrarily taken as a "lytic unit" (LU) (19). The number of LUs/10^6 lymphocytes was calculated.

Limiting Dilution Analysis for CTL Precursor Cells (CTLp)

Limiting dilution assays for determining CTLp frequency were performed as described (20). Limiting numbers of responder cells (C57BL/6) were cultured in round-bottom microplates with an excess of allogeneic tumor cells (10^4 mitomycin-treated P815 cells/well) or DBA/2 spleen cells (irradiated with 1000 R; 10^6/well) in RPMI culture medium supplemented with 25% (vol/vol) secondary mixed lymphocyte culture (MLC) supernatant (21). The cultures were incubated at 37°C in humidified air with 5% CO_2. Twenty-four microcultures were set up per dilution and four to six doubling dilutions were run per group. After 7 days of incubation, target cell lysis was measured by adding ^{51}Cr-labeled P815 target cells (5×10^3/20 μl) to each culture well. The cultures were mixed with an eight-channel micropipetor (Flow Laboratories) and the plates were centrifuged at $30 \times g$ and incubated at 37°C for 5 hrs. Microcultures were scored as positive for cytotoxic activity when the ^{51}Cr released exceeded the mean spontaneous release by three standard deviations. Estimates of CTLp frequency were calculated by an iterative procedure based on maximum likelihood analysis (22).

Preparation of MLC Supernatants

Spleen cells (2.5×10^7) from C57BL/6 mice were mixed with 2.5×10^7 irradiated (1000 R) spleen cells from DBA/2 mice in 20 ml of RPMI culture medium supplemented with 5×10^{-5} M 2-mercaptoethanol and 10% heat-inactivated fetal calf serum. The cultures were incubated upright for 14 days in tissue culture flasks (Corning No. 25100) at 37°C in a humidified atmosphere with 5% CO_2. On the 14th day, the responder cells were harvested, washed, counted, adjusted to 10^6/ml in complete RPMI medium, and restimulated with 10-ml aliquots of irradiated (1000 R) DBA/2 spleen cells (4×10^6/ml) in Falcon flasks (No. 3012). After 24 hrs, the cultures were centri-

fuged and the supernatant was frozen in aliquots at –18°C until use.

Interleukin II

Interleukin II (IL2) was obtained from Collaborative Research Inc, Waltham, MA (lot #81-1162). This preparation was made from Con A rat splenocyte cultures and subjected to ammonium sulfate precipitation and ion exchange chromatography. This IL2 preparation and the MLC supernatants described above were adjusted to the same concentration (2 units/ml) of IL2 activity (23). IL2 activity was determined by Dr. Michael Pallidino (Sloan-Kettering Institute).

RESULTS

The initial experiments were designed to test the effect of ara C on CTL generation in vitro. C57BL/6 mice were injected with ara C (60 and 120 mg/kg) for 6 consecutive days. After a rest period of 24 hrs, the spleens were removed and cultured with mitomycin-treated P815 tumor cells. Table 1 shows the results of the CTL assay performed on the fifth day of culture. Mice injected with saline showed a normal spleen cell recovery and a good CTL response. Mice treated with ara C had approximately a 40% reduction in spleen cellularity. In addition, there was a progressive reduction in the CTL response. In mice treated with ara C, the CTL response was significantly lower in each experiment. The numbers of LU per 10^6 cells and per spleen are also listed; spleen cells from mice treated with ara C showed < 1% the CTL activity of saline-treated controls.

The reduction in CTL activity in spleen cells of ara C-treated mice could be due to direct toxicity to the CTLp or other regulatory cells, as had been true in the case of cyclophosphamide (20, 24–26). The numbers of CTLp in normal and ara C-treated mice were determined by limiting dilution analysis (20,22). Table 2 shows that the total number of CTLp in ara C-treated mice was within the normal range, and in fact, the CTLp frequency in the treated mice was elevated, which probably reflected an enrichment due to the reduction in splenic cellularity. The last column of table 2 shows the actual number of CTLp per spleen in saline- and ara C-treated mice. When splenic cellularity is taken into account, the numbers of CTLp in normal or ara C-treated mice are nearly equal. The values in this table represent a pool of three experiments utilizing 48–72 culture wells per responding cell dose. All limiting dilution experiments were performed in parallel with experiments measuring CTL activity as shown in table 1.

Since ara C has no direct toxicity on CTLp but reduces CTL activity, a regulatory cell other than the CTLp must be affected by this drug. As in the case of cyclophosphamide (26), a helper factor derived from MLC supernatants restored cytolytic capacity to ara C-treated spleen cells (table 3). Experiment 1 shows that reduced CTL activity resulting from ara C treatment was increased by the addition of MLC supernatants to the culture system. Experiment 2 shows similar results, but in addition, a target specificity control (C118 myeloma cells) was added to the ^{51}Cr assay. In this experiment, ara C reduced CTL activity when measured against ^{51}Cr-labeled P815 target cells, and again, this activity was restored by the addition of MLC supernatant. There was no appreciable lysis of the C118 myeloma cells, indicating that specific CTL were generated. The C118 myeloma cells are resistant to natural killer cells but are sensitive to lysis by specific anti-$H\text{-}2^k$ CTL (results not shown).

It was assumed that the active moiety in restoring CTL activity was IL2 as had been previously shown for cyclophosphamide (26,27; unpublished results). Experiment 3 (table 3) shows that when a partially purified source of IL2 was added to the

TABLE 1.—Effect of in vivo ara C administration on CTL generation in vitro

Group*	Spleen cell recovery ($\times 10^6$)†	% specific ^{51}Cr release‡ for lymphocyte:target cell ratios of— 50:1	12:1	6:1	LUs/10^6 spleen cells§	Mean LUs/spleen
Saline	83	41 ± 2.0	35 ± 0.6	30 ± 0.6	21.3	1768
60 mg/kg of ara C	50	30 ± 0.3	25 ± 1.0	21 ± 0.3	2.5	125
120 mg/kg of ara C	48	23 ± 0.9	19 ± 0.9	16 ± 0.3	0.3	14

*C57BL/6 mice were injected with saline or ara C for 6 consecutive days. On the 8th day, spleens were pooled and cultured with mitomycin-treated P815 tumor cells.

†Average No. of nucleated cells/spleens; 2–4 spleens/group.

‡Mean ± SE of triplicate cultures after 5-hr incubation with ^{51}Cr-labeled P815 tumor cells.

§LUs based on the lymphocyte:target cell ratio necessary to obtain 33% lysis.

TABLE 2.—CTLp frequency in control and ara C-treated mice

Group*	Reciprocal mean CTLp frequency (95% confidence interval)†	χ^2‡	Mean No. of recovered cells/ spleen ($\times 10^6$)	Total No. of CTLp/spleen ($\times 10^4$)
Saline	866 (711–1054)	0.62	81.7	9.43
120 mg/kg of ara C	518 (434–619)	1.16	45.3	8.75

*Saline or ara C were administered in 6 daily doses. Spleens were removed the day following final injection and cultured with mitomycin-treated P815 mastocytoma cells for 7 days.

†Determined after 7 days in culture. ^{51}Cr-labeled P815 cells were targets in a 5-hr incubation assay. Data represent pool of 3 experiments.

‡Values are not significant, indicating internal consistency of assay and good agreement between observed dose-response results and those expected, assuming single-hit Poisson model (22).

culture system, CTL activity was significantly increased in cultures of spleen cells derived from ara C-treated mice.

DISCUSSION

Early studies in mice have shown that ara C is effective in the control of recently transplanted or well-established murine leukemias (4,6). Mice receiving repeated administration of ara C (10–50 mg/kg) show an increase in median survival time and a concomitant reduction in tumor load (4). Using the L1210 leukemia system, it was found that the maintenance of relatively constant levels of ara C in the body fluid is a more effective approach for leukemic cell destruction than a single large dose (250–1000 mg/kg). Unfortunately, these studies also show that while most or all leukemic cells theoretically could be eradicated if blood fluid levels of ara C were maintained, the effects of ara C in this regimen were more toxic to normal tissue than in a single high dose (6).

Toxicity by DNA synthesis inhibitors can be either specific or generalized, the latter including marrow and gut destruction (28,29) as well as immunosuppression (30). In regard to immunosuppression, early reports have shown that one dose of ara C (2500 mg/kg; LD10) suppresses the hemagglutinin response of mice to sheep rbcs when the drug is given simultaneously with or up to 72 hrs after antigen (31). Another study demonstrated that selective tolerance to human erythrocytes could be induced in rats by high doses of ara C (1000 mg/kg/day for 5 days) (32).

The purpose of this study was to evaluate the effects of repeated administration of ara C on the development of CTL. Inhibition of CTL activity was not due to the elimination of CTLp but apparently resulted from the removal of a second signal needed for CTL development. Cytotoxic potential was re-

TABLE 3.—Restoration of CTL activity in spleen cells of ara C-treated mice

Experiment No.	Group*	No. of cells recovered/spleen ($\times 10^6$)	Helper factor†	% ^{51}Cr release‡		LUs/10^6 cells§	Mean LUs/spleen
				P815	C118		
1	Saline	81	–	55 ± 0.7	ND	20.6	1669
			+	53 ± 1.2	ND	21.5	1742
	120 mg/kg of ara C	38	–	17 ± 1.2	ND	1.0	38
			+	41 ± 4.2	ND	12.3	467
2	Saline	81	–	79 ± 1.5	9 ± 0.6	47.1	3815
	120 mg/kg of ara C	50	–	34 ± 0.3	1 ± 0.1	10.1	505
			+	66 ± 0.8	4 ± 0.3	39.2	1960
3	Saline	71	–	76 ± 2.9	ND	42.3	3003
	120 mg/kg of ara C	20.5	–	19 ± 0.9	ND	2.9	59
			+	59 ± 1.2	ND	24.7	506

*C57BL/6 mice were injected with saline or ara C for 6 consecutive days. On the 8th day, spleens were pooled and cultured with mitomycin-treated P815 tumor cells.

†MLC supernatant (50% vol/vol) in experiments 1 and 2; IL2 in experiment 3.

‡Lymphocyte:target cell ratio of 25:1. Values are mean ± SE of quadruplicate cultures; 2–4 spleens/group. ND = not done.

§LUs based on the lymphocyte:target cell ratio necessary to obtain 33% lysis of P815 target cells.

stored to drug-treated lymphocytes by sources of IL2 (table 3). It therefore seems that ara C removes a helper cell required for the production of IL2. Similar results were obtained after the administration of high doses of cyclophosphamide (24–26). In these previous studies, it was shown that cyclophosphamide administration inhibited the CTL response of murine spleen cells by removing a helper cell (25) and helper factor (26). We are now studying several sources of purified IL2 and are quantifying the actual number of units of IL2 necessary to restore CTL activity in both systems.

It is possible that inhibitors of DNA synthesis such as ara C and cyclophosphamide selectively remove regulatory cells (24,25) and leave CTLp intact (20). This being the case, it may be advantageous in the treatment of neoplasia to administer supportive therapy (helper factor) along with the cytotoxic drug to "rescue" or protect the immune system. Rescue therapy in vivo after cyclophosphamide administration has proven efficacious (27) and may also be applicable with ara C.

Although we have focused here only on the rescue of the CTL system, it may be possible, in the future, to employ methods of protecting both humoral and cell-mediated immunity as well as gut- and marrow-associated toxicity. If these major areas of the host's tissue can be selectively protected during chemotherapy, a greater degree of tumor toxicity may be achieved with the use of higher, more effective doses of antineoplastic agents.

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Randomized Phase II Comparison of Methotrexate With or Without L-Asparaginase in Non-Small Cell Cancer of the Lung [1,2]

Brief Reports

Seth A. Rudnick,* Eric Neijstrom, Robert L. Capizzi, David Delaney, and Mark Kirsch [3]

SUMMARY

Since conventional doses of methotrexate (MTX) have been reported to be useful in non-small cell cancer of the lung (NSCLC), a randomized trial was designed to test whether L-asparaginase (L-asp) might improve its efficacy and allow patients to tolerate larger doses of MTX. Twenty-three patients (18 men and five women) were randomized to receive an 8-week course of escalating doses of MTX weekly (12 patients) or escalating doses of MTX every 10 days followed by L-asp (10,000 IU) 24 hours later (11 patients). The starting MTX dose was 40 mg/m² in both groups followed by radiation therapy to the primary site. If there was no response to the initial MTX with or without L-asp, patients were then switched to a combination of VP-16-213, doxorubicin, and mitomycin. All 23 patients had stage III disease and a performance score of 0–3. Sixteen of the 23 patients had adenocarcinoma. Each group was balanced according to sex, age, and performance score. Among the 12 patients treated with MTX alone, there were two partial responses. There were no responses in the 11 patients treated with MTX and L-asp. In the MTX alone group, the average maximally tolerated dose of MTX was 50 mg/m² (range, 40–80). In the MTX and L-asp group, the average maximally tolerated dose was 90 mg/m² (range, 40–180) ($P < 0.05$). L-Asp allowed a higher dose of MTX to be administered without leukopenia or mucositis; however, nausea, vomiting, and diarrhea were considerably more frequent and severe in the MTX and L-asp group. This resulted in premature discontinuation of the MTX and L-asp treatment in six patients. Two patients developed documented thrombophlebitis and one developed pulmonary embolus while receiving MTX and L-asp. The median survival times of the MTX and MTX and L-asp groups were 190 and 160 days, respectively ($P > 0.05$). In summary, MTX plus L-asp is apparently not useful and is highly toxic in NSCLC, in contradistinction to acute lymphocytic leukemia and breast cancer.

[Cancer Treat Rep 66:541–544, 1982]

Since most patients with non-small cell cancer of the lung (NSCLC) have unresectable disease, the development of effective chemotherapy regimens, as well as the evaluation of new antineoplastic agents, remains an important goal of oncology research. Currently, few drugs, either alone or in combination, have led to prolonged survival. Among the partially active single agents, methotrexate (MTX) produces a response rate of 20%–25% (1,2).

L-Asparaginase (L-asp) has been shown to improve the therapeutic index of MTX (3), and the combination has apparent schedule-dependent synergistic effects in murine (4) and human (5) acute lymphocytic leukemia (ALL). The superior therapeutic effects of the combination have been verified in larger trials in ALL refractory to conventional doses of MTX (6–8). In addition, this combination has been effective in patients with breast cancer who have relapsed while receiving therapy with combinations containing MTX (9). In view of this activity, a randomized trial comparing escalating doses of MTX alone versus escalating doses of MTX followed by L-asp was conducted. The purpose of the trial was not only to assess the relative therapeutic effects of the drugs but also to determine if L-asp protects the normal bone marrow and gas-

[1]Received Feb 23, 1981; revised Sept 4, 1981; accepted Sept 29, 1981.

[2]Supported in part by the R. J. Reynolds Medical Oncology Trust Fund. S. A. Rudnick is the recipient of a National Cancer Institute Preventive Oncology Academic Award (1-K07-CA-00722).

[3]Departments of Medicine, Radiology, and Epidemiology, The University of North Carolina, Chapel Hill.

*_Reprint requests to:_ Seth A. Rudnick, MD, Schering-Plough Corp, 60 Orange St, Bloomfield, NJ 07003.

trointestinal tract from the toxic effects of MTX as it does in patients with ALL (3).

This study also incorporated the use of irradiation after the MTX with or without L-asp therapy and, depending on initial response, the use of VP-16-213, doxorubicin, and mitomycin (VAM). These latter drugs have demonstrable, though limited, activity in NSCLC (10–12) and were utilized regardless of the earlier randomization.

MATERIALS AND METHODS

Twenty-three patients (18 men and five women) with a histologically or cytologically confirmed diagnosis of NSCLC were entered in the study from August 1, 1979, to June 30, 1980 (fig 1). All patients had stage III disease and, therefore, were not considered surgical candidates. An ambulatory Eastern Cooperative Oncology Group performance status (PS) of 0–3 was required for entry in the study. All patients had clinically detectable disease evaluable either by physical examination or by roentgenographic techniques. No patient had received prior chemotherapy; however, four had received irradiation to their lung primary before entering the study.

Pretreatment evaluation consisted of a complete medical history and physical examination, cbc, liver function tests, BUN, creatinine, chest roentgenogram, prothrombin time, partial thromboplastin time, and thrombin clotting time.

Liver and spleen scans, bone scans, computerized tomographic head or brain scans, and bone marrow aspirate and bilateral biopsies were obtained as part of the staging evaluation. All but six of the potential 125 staging procedures were performed, four due to patient refusal. Gated blood pool scanning was performed in 18 of 23 patients, all with ejection fractions in the range of 62%–88%. All creatinine clearances were > 55 ml/minute (range, 56–140).

Responses were evaluated every 4 weeks. A complete response (CR) was defined as the disappearance of all clinical and radiologic evidence of disease, and a partial response (PR) was defined as a decrease of ≥ 50% in the product of two perpendicular diameters for measurable disease and > 70% reduction in the total area of evaluable disease, with no progression of disease in any other area, lasting for at least 4 weeks. Subjective improvement alone

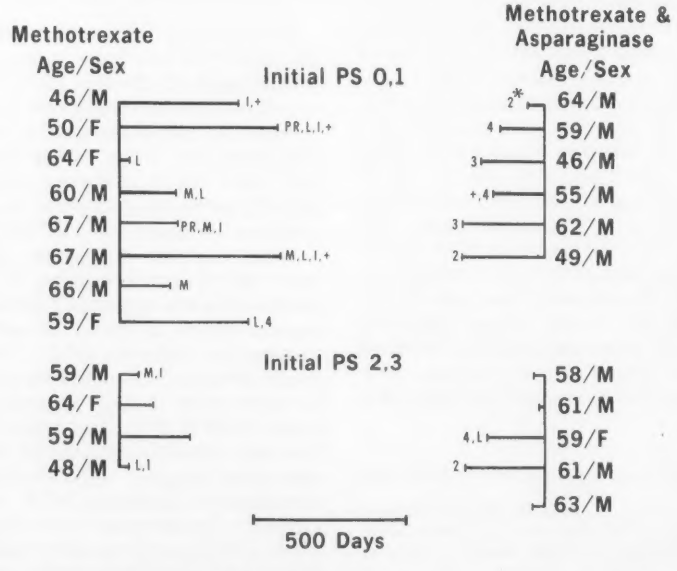

FIGURE 1.—Response, toxicity, and survival by treatment and by PS. Bar length = survival time (+ = censored); *1, 2, 3, 4 = maximum grade of nausea, vomiting, and diarrhea during any cycle; L = leukopenia < 4000 cells/mm³ at nadir; M = mucositis. Figure modified from a suggestion by Zelen.

was not considered to be a response. No patient required a repeat scan to assess response. Survival was calculated from the date when MTX was first administered.

Patients were stratified on the basis of PS and the presence or absence of metastatic disease beyond the thorax. They were then randomized to receive doses of either 40 mg/m² of MTX alone with 5 mg/m² escalating doses each week (group 1) or MTX followed 24 hours later by L-asp (with 50% MTX escalation) every 10 days (group 2) to MTX tolerance. This initial portion of the study was carried out over 8 weeks.

After completing this initial phase of therapy, patients not previously treated with radiation therapy were given 3000 rads to the primary, mediastinum, and supraclavicular nodes if palpably involved. Responding patients were maintained on MTX (with or without L-asp) for a maximum of 1 year and then switched to VAM. If patients failed earlier than the initially proposed 8-week course of therapy, irradiation was not delayed. If irradiation was not possible, or if disease progression or disease stabilization had occurred, patients were immediately treated with VAM. Written informed consent was obtained from all patients with an explanation of potential toxicity and alternative therapy.

An early stopping point was constructed in the trial so that an evaluation was to be made after the first 11 MTX and L-asp patients were treated. If no responses were seen, the 95% confidence limit for activity would not be > 30%, thus suggesting no greater activity than MTX or other single agents alone (13).

RESULTS

Staging

Staging evaluation revealed that two of 19 (11%) patients had a positive bone marrow aspirate or biopsy. Four of 23 (17%) patients had metastases to the liver. Nine of 22 (41%) patients had positive bone scans, and five of 22 (23%) had positive computerized tomographic head scans. Four of the five patients with abnormal computerized tomographic scans were asymptomatic with neurologic examinations.

Response (Fig 1)

Two of the 12 patients treated with MTX alone (group 1) achieved a PR, while none of the 11 patients receiving MTX and L-asp (group 2) sustained a response. Three of the group 2 patients, however, achieved a PR after being treated with radiation therapy. There were no additional responses obtained with irradiation in the MTX group. The maximally attained MTX dose was clearly higher in the MTX and L-asp group, ie, 50 versus 90 mg/m² ($P < 0.05$, $T = 2.12$).

Of 12 patients who eventually were treated with VAM, there was one additional responder (PR) who had been withdrawn from MTX because of toxic reaction and who was not irradiated because of extensive pulmonary involvement. Two previously responding patients, one from MTX (CR) and the other from irradiation (PR), had their responses maintained with VAM. The patient with a CR is alive at 500+ days, having been treated with MTX for 1 year, and the patient who achieved a PR with radiation therapy has been maintained on VAM for 500+ days. Overall, there have been six responses (MTX, two; irradiation, three; VAM, one), and all occurred in patients with adenocarcinoma. The median survival for group 1 patients was 90 days versus 160 days for group 2 patients (log-rank, $P > 0.05$).

Toxicity

Patients in group 2 (fig 1) received a significantly higher average dose of MTX ($P \leq 0.05$), but toxic effects consisting of nausea, vomiting, and diarrhea were poorly controlled in eight patients and required withdrawal of the drugs in three. In contrast, six patients in group 1 had nausea and vomiting but five were well-controlled by standard antiemetics, and only one required discontinuation of MTX. Mucositis occurred only in those patients receiving MTX alone. Neither group experienced thrombocytopenia (< 100,000 cells/mm³); however, six patients in group 1 developed leukopenia as compared with one patient in group 2 ($P \leq 0.05$). Two patients had deep venous thrombophlebitis and one had pulmonary embolus while receiving MTX and L-asp.

DISCUSSION

Approximately 95% of inoperable patients with NSCLC will be dead in 12 months, with median survival times ranging from 3 to 6 months. Recent studies (14–18) have demonstrated response rates of 36%–58% for combination chemotherapy in squamous cell carcinoma or adenocarcinoma of the lung. Responders have had significantly longer median survival times (12–15 months) compared to nonresponders (5–6 months). However, few multi-institutional cooperative groups have been able to show

response rates above the 16%–25% reported for single-agent therapy.

In an attempt to maximize the single-agent response rate by increasing the dose while limiting toxicity, MTX with L-asp was compared to MTX alone. No responses were observed with this combination of drugs, despite the higher doses of MTX administered. Overall, only two of 23 (9%) patients responded to MTX, a lower figure than usually quoted for this single agent. Although L-asp has not been reported to have activity in bronchogenic carcinoma, little evidence has previously existed for adverse effects in bronchogenic carcinoma, through either coagulation or the gastrointestinal tract. In fact, similar regimens have shown efficacy without excess toxicity in patients with ALL (5) and breast carcinoma (9).

Not surprisingly, abnormalities occurred that may have been due to unmasking effects of the L-asp on the coagulation cascade in patients with lung cancer. In a recent report, Priest et al noted three cerebral infarctions in patients with ALL receiving L-asp therapy and described abnormalities in fibrinogen, prothrombin time, partial thromboblastin time, and antithrombin III (19). While our study demonstrates no statistically significant differences between the L-asp- and non-L-asp-treated groups, the development of one episode of deep venous thrombophlebitis and one of pulmonary embolus while receiving L-asp is of grave concern in considering further use of this drug. Other differential toxic effects were more clearly noted and included mucositis and leukopenia in the MTX group. This suggested that "rescue" of these organs by L-asp may have occurred. However, this rescue was at the cost of severe gastrointestinal toxicity with the MTX and L-asp combination. Thus, in addition to the lack of efficacy of MTX and L-asp, probably less than that of MTX and certainly no better than a 30% projected response rate, we believe the unacceptable toxicity of this combination precludes its further use in lung cancer trials.

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Response of Hypereosinophilic Syndrome to 6-Thioguanine and Cytarabine[1]

Brief Reports

Daryl L. Eakin, David P. Gill, and Gary B. Weiss[2,*]

SUMMARY

A patient with hypereosinophilic syndrome had progression of disease and severe toxic effects while receiving a conventional regimen of prednisone and hydroxyurea. Treatment with 6-thioguanine and cytarabine produced an excellent response and may be a useful regimen in those patients who have unacceptable toxic effects or fail to respond to conventional therapy for hypereosinophilic syndrome.

[Cancer Treat Rep 66:545–547, 1982]

Hypereosinophilic syndrome is a disease of unexplained and persistent peripheral blood eosinophilia, organ infiltration, and a progressively unfavorable clinical course (1). Although controversy exists as to whether it is a leukemic process, published data suggest that when this disorder is untreated, it has a range of mortality similar to that of adult leukemia (2). Patients with wbc counts > 100,000/mm^3 have a poor prognosis (9-month survival rate of 25%) (2). Survival may be markedly prolonged by treatment (3-year mortality of 4%); hydroxyurea appears to be the treatment of choice in those patients with hypereosinophilic syndrome who have failed to respond to prednisone (3).

This paper reports a patient with hypereosinophilic syndrome who had progressive organ infiltration and clinical deterioration despite marked suppression of peripheral blood counts while he was receiving hydroxyurea. A favorable and prolonged response was obtained with the combination of 6-thioguanine and cytarabine, a regimen that has not been described in the treatment of hypereosinophilic syndrome.

[1]Received May 18, 1981; revised July 29, 1981; accepted Aug 7, 1981.

[2]Division of Hematology and Oncology, Department of Internal Medicine, University of Texas Medical Branch, Galveston.

**Reprint requests to:* Dr. Gary B. Weiss, Division of Hematology and Oncology, MW-404, University of Texas Medical Branch, Galveston, TX 77550.

CASE REPORT

A previously healthy 38-year-old man complained of a 4-week history of symptoms consistent with congestive heart failure in February 1979. Physical examination revealed numerous small cervical and epitrochlear nodes, cardiomegaly with a murmur of mitral insufficiency, pitting pretibial edema, and a palpable spleen tip. The patient had no history or physical findings of angioedema. Relevant laboratory data included: hematocrit, 29%; wbc count, 17,000/mm^3, with 17% segmented neutrophils, 6% band forms, 10% lymphocytes, 3% monocytes, 54% mature eosinophils, and 2 nucleated rbcs/100 wbcs; reticulocyte count, 4%; platelet count, 127,000/mm^3; gamma globulin, 2.7 g/dl (polyclonal); uric acid, 8.4 mg/dl; rheumatoid arthritis (latex) titer, 1:40; antinuclear antibody, negative; multiple stool examinations, negative for ova and parasites; and a vitamin B_{12} level > 2000 pg/ml. A muscle biopsy was normal. Bone marrow aspiration and biopsy revealed a cellularity of 95%–100%. The myeloid-to-erythroid ratio was 20:1, with 60% of the marrow cellularity being comprised of eosinophilic precursors. Myeloblasts were not increased, but reticulin was moderately increased. The chromosomal karyotype was normal.

The patient's heart failure responded to digoxin and furosemide. However, 1 month after he was first seen, his total wbc count had increased to 27,000/mm^3, with 51% eosinophils; immature granulocytes were present on the peripheral blood smear. Oral prednisone (60 mg/day) was begun, but the total wbc count continued to increase (fig 1). Eosinophils consistently comprised 40%–60% of the peripheral wbc count. Oral hydroxyurea (2.0 g/day) and a single iv injection of vincristine (2.0 mg) were then given (3). There was a prompt decrease in the total wbc count, but neutropenia (1100 neutrophils/mm^3) and thrombocytopenia (11,500 platelets/mm^3) developed simultaneously with progressive hepatosplenomegaly and ascites, which were believed to be secondary to eosinophilic infiltration and not to congestive heart failure. Due to the patient's de-

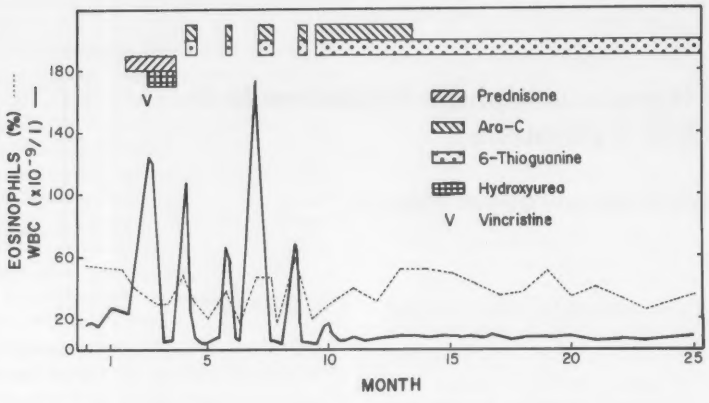

FIGURE 1.—Serial wbc counts and percent eosinophils during 25 mos of varied treatments (*see* text for dosages). Treatment during Months 4–9 consisted of 4 high-dose courses of 6-thioguanine and cytarabine (ara-C) (*see* text). Outpatient treatment has been given since Month 9 from diagnosis.

teriorating clinical condition, it was felt that a rapid reduction in parenchymal organ infiltration was necessary. A review of the literature failed to suggest a successful alternative regimen. Therefore, a regimen for leukemia described by Clarkson et al (4) was begun. This consisted of 3.0 mg/kg of cytarabine iv and 2.5 mg/kg of 6-thioguanine orally every 12 hours. This regimen was continued until the total peripheral wbc count decreased to 6000–8000/mm^3. Severe neutropenia was not experienced; however, there was severe thrombocytopenia (8500 platelets/mm^3). This was repeated four times at intervals of 3–6 weeks. Marrow recovery was allowed prior to repeating subsequent courses. This recovery was accompanied by increasing hepatosplenomegaly, which rapidly resolved with each treatment. On two occasions, treatment was complicated by clinical evidence (prolonged bleeding from bone marrow and venipuncture sites) and laboratory evidence (fibrinogen, 120 mg/dl; fibrin degradation products > 40 μg/ml; factor V < 0.1 unit/ml; factor X < 0.05 unit/ml; and factor VIII < 0.01 unit/ml) of disseminated intravascular coagulation, which responded to heparinization (10,000–24,000 units/day) and replacement of clotting factors (cryoprecipitate and fresh-frozen plasma).

Outpatient treatment was begun 9 months after the patient was first seen; he received 120 mg of 6-thioguanine orally three times per week and 100 mg/wk iv of cytarabine. Subsequent modifications were made on the basis of wbc and platelet counts, and cytarabine was discontinued 4 months later. The patient is currently maintained with 40 mg of 6-thioguanine given orally three times per week. During this therapy, the hepatosplenomegaly has resolved and the ascites has decreased dramatically. The patient has not been hospitalized since the beginning of this regimen. Continued use of digoxin and furosemide has been necessary to control congestive heart failure. Eosinophilia persists (1500–3000 eosinophils/mm^3). The patient is currently active and experiences minimal physical limitations 29 months after the diagnosis of hypereosinophilic syndrome was made.

DISCUSSION

This patient's course is similar to previously published cases of hypereosinophilic syndrome (1–3). However, hydroxyurea produced severe toxic effects in this patient while his disease progressed. It seems unlikely that a lower dose of hydroxyurea would have been more effective (less severe toxic effects and a greater reduction of disease with longer treatment), as the disease progressed while the patient was receiving the higher dose. Intermittent treatment with high-dose 6-thioguanine and cytarabine resulted in a rapid lowering of the total wbc count and clinical improvement but was associated with marked fluctuations in wbc counts after each treatment. The rapid cell death noted with this regimen was probably the etiology of the patient's disseminated intravascular coagulation, a complication not previously reported during the treatment of hypereosinophilic syndrome. Changing to low-dose

continuous treatment with 6-thioguanine and cytarabine has resulted in excellent control of the patient's symptoms and wbc counts. Complete remission has not been achieved; this is in agreement with the experience of other investigators (3). The decrease in eosinophil count was greater than the decrease in total wbc count with the hydroxyurea and prednisone and with each of the four intermittent high-dose courses of 6-thioguanine and cytarabine (fig 1). Treatment with 6-thioguanine and cytarabine may be a useful regimen in those patients who have unacceptable toxic effects or who fail to respond to conventional therapy for hypereosinophilic syndrome.

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Radiation Therapy Combined With Intra-arterial 5-FU Chemotherapy for Treatment of Localized Adenocarcinoma of the Pancreas: A Southwest Oncology Group Study[1,2]

Brief Reports

Joseph D. McCracken,[3,*] Marvin Olson,[4] A. B. Cruz, Jr,[5] Lawrence Leichman,[6] and Noboru Oishi[7]

The Southwest Oncology Group has conducted a pilot study of localized, unresectable pancreatic cancer utilizing direct celiac artery infusion of 5-FU combined with radiation therapy, followed by systemic mitomycin and 5-FU maintenance therapy, and has evaluated the effect on survival and the toxic effects of this multimodality program.

The impetus for this pilot work was a recently completed Southwest Oncology Group study (1) that utilized systemic 5-FU plus methyl-CCNU combined with 6000 rads in treating localized pancreatic cancer. The median survival for all patients in this study was 38 weeks. Most patients experienced moderate to severe toxic reactions, requiring interruption of treatment and dose reductions. The positive survival benefits appeared marginal compared to the toxicity encountered when full-dose radiotherapy and chemotherapy were utilized together. In a number of studies (2,3), intra-arterial chemotherapy through hepatic artery or intra-aortic infusions has yielded improved responses and survival in patients with cancer of the colon metastatic to the liver. Toxic reactions are uncommon and the placement of a catheter via percutaneous technique is easily accomplished with minimal morbidity. Attempts at control of localized pancreatic cancer by intra-arterial infusion using similar techniques have not been extensively evaluated.

The study we now report explored lower dose radiotherapy (5000 rads) with sequential regional intra-arterial chemotherapy to determine if such a program would yield similar or improved survival with less toxicity.

MATERIALS AND METHODS

Patient eligibility included no age or sex restriction, histologic confirmation of adenocarcinoma of the exocrine pancreas, tumor margins outlined by radiopaque clips to create a port size ≤ 225 cm^2 (approximately 15 × 15 cm), wbc count at least 4000/mm^3, platelet count at least 100,000/mm^3, established wound healing, and a clinical situation good enough to allow initiation of therapy between 15 and 42 days after surgery. Patients with disease outside of the 225-cm^2 port or with metastatic disease were ineligible for this study.

Under fluoroscopic control with angiographic confirmation, a catheter was placed, either by the transbrachial or transfemoral approach, into the celiac artery or other artery identified at the time of surgery or by angiogram to be the principal blood supplier for the tumor. 5-FU at a dose of 1000 mg/m^2 in 1000 ml of 5% dextrose in water was given in a 24-hour infusion daily for 5 days (Days 1–5). Heparin (1000–2000 units) was added to the solution to prevent catheter clotting.

On Days 8–24, 2500 rads was given in 12–13 fractions to the tumor bed. On Days 29–33, a second intra-arterial 5-day course of 5-FU identical to the first course was administered. On Days 36–51, a second 2500 rads of radiation therapy was given, after which maintenance chemotherapy was begun.

Patients were treated with either a cobalt-60 unit or an X-ray beam obtained from X-ray generators operating at a peak energy ≥ 2 MeV. The minimum source-skin distance used was 80 cm. The patients were treated with parallel opposing anterior and

[1]Received May 26, 1981; revised Aug 28, 1981; accepted Sept 18, 1981.

[2]Supported in part by Public Health Service grants CA-22411, CA-14028, CA-22433, CA-12014, and CA-16943 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Oncology Service, Brooke Army Medical Center, Fort Sam Houston, TX.

[4]Radiation Therapy Department, University of Texas, Galveston.

[5]Department of Surgery, University of Texas, San Antonio.

[6]Department of Oncology, Wayne State University Medical Center, Detroit, MI.

[7]Department of Oncology, Cancer Center of Hawaii, Honolulu.

**Reprint requests to:* Dr. J. McCracken (7861), Southwest Oncology Group, Operations Office, 4450 Medical Dr, San Antonio, TX 78229.

TABLE 1.—Induction toxicity

	No. of patients by toxicity code					
	0	1	2	3	4	5
Leukopenia*	13	4	1	0	0	0
Nausea and vomiting	9	4	4	1	—	—
Thrombocytopenia†	17	0	1	0	0	0
Diarrhea	16	1	1	0	—	—

*Toxicity codes (wbcs/mm^3): 0 = > 4000; 1 = 3000–4000; 2 = 2000–3000; 3 = 1000–2000; 4 = < 1000; and 5 = treatment-related death.

†Toxicity codes (platelets/mm^3): 0 = > 100,000; 1 = 75,000–100,000; 2 = 50,000–75,000; 3 = 25,000–50,000; 4 = < 25,000; and 5 = treatment-related death.

posterior fields or with any other multiple-field arrangement as deemed appropriate to avoid high doses to critical organs. The size of the radiotherapy portals was determined by the clips around the tumor placed by the surgeons during exploratory laparotomy.

Maintenance chemotherapy was scheduled to begin 2 weeks after completion of the induction therapy; it consisted of 15 mg/m^2 of mitomycin every 8 weeks by single iv bolus injection and 1000/mg/m^2/day of 5-FU mixed in 1000 ml of 5% dextrose in water given by 96-hour continuous iv infusion and repeated every 4 weeks.

RESULTS

Nineteen patients were entered in this study. One patient was judged to be unevaluable because of protocol violations. The median survival for all patients entered in this study was 27 weeks. No patient experienced more than minimal hematologic toxic reactions in the initial phase of the treatment program (table 1).

Celiac artery catheterization was easily accomplished in all 18 evaluable patients. Adherence to the chemotherapy and radiotherapy schedule along with minimal GI toxicity suggested our regimen to be quite feasible and practical. We encountered severe hematologic toxicity (table 2) during the maintenance therapy, and one patient experienced a fatal granulocytopenia. As a result of this, the maintenance dose of mitomycin was lowered from 15 to 10 mg/m^2. No further incidence of severe or life-threatening toxic reactions was noted. In one patient who died from sepsis, no tumor was identified in the pancreas at autopsy. However, for ten of the 18 patients, followup physical examination, X-ray study, computerized tomographic scan, or ultrasound study demonstrated inadequate local control with evidence of disease progression within the radiotherapy port.

DISCUSSION

Intra-arterial 5-FU when combined with radiotherapy appears to be a well-tolerated program with excellent patient acceptance. Recently, the Gastrointestinal Tumor Study Group (4) reported results of localized pancreatic carcinoma treatment in three randomized groups. They noted significantly better median survival in patients receiving 4000 rads plus 5-FU and 6000 rads plus 5-FU (35 and 28 weeks, respectively) than in the group receiving 6000 rads alone (17 weeks). In our prior study (1) utilizing 6000 rads of radiotherapy combined with concurrent methyl-CCNU plus 5-FU, patients had a median survival of 38 weeks. Unfortunately, our less aggressive radiotherapy and intra-arterial chemotherapy program has not resulted in clearly improved survival over more aggressive programs (5–7) using combination chemotherapy and higher doses of radiation therapy. Because of our high local

TABLE 2.—Maintenance toxicity

	No. of patients by toxicity code*					
	0	1	2	3	4	5
Leukopenia	10 (5)	0	2	4	1	1
Thrombocytopenia	9 (4)	5 (1)	2	1	2	0
Nausea and vomiting	11 (5)	4	2	1	—	—

*Values in parentheses = No. of patients treated with 10 mg/m^2 of mitomycin post-amendment. *See* table 1 for identification of hematologic toxicity codes.

failure rate, it appears that radiotherapy doses of 5000 rads are inadequate for local control in most patients. Because of the lack of hematologic toxicity encountered with this regimen, higher doses of radiotherapy can be utilized and may deserve future study.

Although pharmacokinetic studies were not done, our attempt at intra-arterial therapy may have delivered less than desired levels of chemotherapy to the tumor and achieved blood levels only slightly higher than peripheral vein infusions because of the variable arterial blood supply of the pancreas (up to 40% of patients have branches of the inferior pancreaticoduodenal artery arising from the superior mesenteric artery) (8). Hafstrom et al (9) have observed no objective tumor remissions or subjective improvement in 19 patients treated with regional intra-arterial 5-FU infusion. Because of these observations, the additional patient care costs for inpatient hospitalization for arterial line placement, and the morbidity associated with intra-arterial lines, we do not plan to continue this program.

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Extensive Adenocarcinoma and Large Cell Undifferentiated Carcinoma of the Lung Treated With 5-FU, Vindesine, and Mitomycin (FEMi): A Southwest Oncology Group Study[1,2,3]

Brief Reports

Thomas P. Miller,[4] James K. Weick,[5] Petre N. Grozea,[6] and David A. Carlin[4,*]

SUMMARY

Sixty-three previously untreated patients with metastatic non-small cell lung cancer (40 patients with adenocarcinoma and 23 with large cell undifferentiated carcinoma) were treated with combination chemotherapy consisting of 5-FU (300 mg/m²) given as an iv bolus on Days 1–4 and vindesine (3 mg/m²) and mitomycin (10 mg/m²), both given as an iv bolus on Day 1 of each treatment course (FEMi). FEMi was repeated at 3-week intervals for three treatment courses and thereafter at 6-week intervals until disease progression. Major objective responses were seen in 13 of the 63 patients (21%). Minor responses were seen in an additional 12 patients (19%). The median survival for all patients was 23 weeks and for responding patients was 38 weeks. The pretreatment performance status had a significant effect on both the response rate and survival time. Patients having an initial Karnofsky performance score ≥ 70% had a 54% response rate, with a median survival of 42 weeks for responding patients. FEMi was well-tolerated: 18 patients (29%) did not have any side effects and only five (8%) experienced vomiting.

[Cancer Treat Rep 66:553–556, 1982]

The most commonly employed drug combinations for the treatment of advanced non-small cell lung cancer contain doxorubicin and cyclophosphamide. In 1978, a drug regimen containing neither doxorubicin nor cyclophosphamide was developed utilizing 5-FU, vincristine, and mitomycin (FOMi) (1). The FOMi regimen was developed by choosing active single agents specific for adenocarcinoma and large cell undifferentiated carcinoma of the lung. Furthermore, drugs were chosen to offer a potentially non-cross-resistant combination for use with, or after treatment with, the standard doxorubicin and cyclophosphamide-containing combinations.

Since 1978, vindesine has been extensively tested in phase II trials and has been shown to be an active single agent in the treatment of advanced non-small cell lung cancer, with an overall objective response rate of 28% in 177 patients (2–7). In June 1979, vindesine was substituted for vincristine in the FOMi regimen. The new drug combination, FEMi, has since been tested within the Southwest Oncology Group (SWOG). This paper describes the SWOG experience using the FEMi regimen as initial treatment for 63 patients with advanced adenocarcinoma and large cell undifferentiated carcinoma of the lung.

PATIENTS AND METHODS

Between June 1979 and October 1980, 63 patients with biopsy-proven adenocarcinoma and large cell undifferentiated carcinoma of the lung were treated with the FEMi drug regimen. All patients had advanced disease and were previously untreated with chemotherapy. Patients who had received prior irradiation with curative or debulking intent were eligible for the study only if the disease was shown to have progressed following completion of radiation therapy. Concomitant radiation therapy was allowed only for the relief of pain and to treat brain

[1]Received July 20, 1981; revised Oct 7, 1981; accepted Nov 3, 1981.

[2]Supported by Public Health Service grants CA-17094, CA-13612, CA-16957, CA-04919, CA-12014, and CA-16943 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and in part by funds from Eli Lilly and Co, Indianapolis, IN.

[3]Vindesine (Eldesine) was supplied by Eli Lilly and Co, Indianapolis, IN.

[4]Department of Internal Medicine, University of Arizona Cancer Center and Tucson Veterans Administration Medical Center.

[5]The Cleveland Clinic Foundation, OH.

[6]Oklahoma Medical Research Foundation, and the University of Oklahoma Health Sciences Center, Oklahoma City.

**Reprints requests to:* Southwest Oncology Group (SWOG-7971), Operations Office, 4450 Medical Dr, San Antonio, TX 78229.

metastases. The median age for FEMi-treated patients was 60 years (range, 44–76). Other pretreatment patient characteristics are listed in table 1.

FEMi consisted of 5-FU (300 mg/m²) given as an iv bolus on Days 1–4, and vindesine (3 mg/m²) and mitomycin (10 mg/m²), both given as an iv bolus on Day 1 of each treatment course. FEMi was repeated at 3-week intervals for three treatment courses and thereafter at 6-week intervals until disease progression. Appropriate reductions in the dose-time rate were made if significant myelosuppression occurred.

A complete response (CR) was defined as the disappearance of all clinical evidence of tumor. A partial response (PR) was defined as a ≥ 50% decrease in the sum of the products of two perpendicular tumor diameters for all evaluable lesions. A minor response was defined as an objective improvement, less than that defined for a PR. Progressive disease was any increase in tumor size, the appearance of new lesions, or no evidence of response after two courses of FEMi. The duration of response was calculated as disease-free survival from the time of initiation of chemotherapy until disease progression. Survival was calculated from the time of beginning treatment with FEMi until death or until the date of last followup using the actuarial method of Kaplan and Meier. Comparisons were made using Gehan's modification of the Wilcoxon test and the log-rank method. Four patients died within 3 weeks of starting treatment and two were lost to followup. These patients were included in the analyses and are considered to be treatment failures. The toxicity of treatment is graded according to SWOG criteria. The degree of myelosuppression was determined on Day 1 of each treatment course.

TABLE 1.—Pretreatment characteristics of 63 patients treated with FEMi

Characteristic	No. of patients (%)
Sex	
Male	51 (81)
Female	12 (19)
Histology	
Adenocarcinoma*	40 (63)
Large cell undifferentiated carcinoma	23 (37)
Stage	
III M_0	3 (5)
III M_1	60 (95)
Karnofsky PS (%)	
90–100	15 (24)
80–89	9 (14)
70–79	17 (27)
60–69	13 (21)
< 60	9 (14)
Pretreatment weight loss	
None	16 (25)
< 10%	12 (19)
> 10%	23 (37)
Unknown	12 (19)
Sites of initial disease involvement	
Primary lung	56 (89)
Contralateral lung	19 (30)
Lymph nodes	43 (68)
Bone	30 (47)
Skin	10 (16)
Pleura	10 (16)
Central nervous system	9 (14)
Liver	9 (14)
Other	5 (8)
Prior treatment	
Radiation	26 (41)
Surgery	10 (16)
Chemotherapy	0 —

*Includes adeno-squamous carcinoma (1) and alveolar cell carcinoma (1).

RESULTS

The results of treatment of 63 patients with FEMi are summarized in table 2. Objective responses (minor response plus PR plus CR) were seen in 25 of 63 patients (40%). Major responses (PR plus CR) were observed in 13 of 63 patients (21%). For responding patients the median disease-free interval was 17 weeks. The cell type did not influence the response rate: 15 of 40 patients (38%) with adenocarcinoma responded and ten of 23 patients (43%) with large cell undifferentiated carcinoma responded to FEMi. The pretreatment PS was an important prognostic factor determining response to FEMi. Patients with a Karnofsky PS < 70% seldom responded, whereas most of the patients having a PS ≥ 70% responded (table 2). Further selection of patients for treatment based on initial PS would result in a 67% response rate in 23 patients with a PS ≥ 80%. There were no responses in nine patients who had a PS < 60%. In this study pretreatment weight loss did not predict response to FEMi (table 2).

The median survival for the entire group of patients was 23 weeks, with a median followup of 60 weeks. Patients who objectively responded to FEMi lived significantly longer than nonresponders (median survival, 38 vs 21 weeks; $P = 0.02$). Thirteen patients with major responses (PR plus CR) lived a median of 44 weeks, whereas 12 patients with minor responses lived a median of 24 weeks ($P = 0.34$). If only patients who began FEMi with a PS ≥ 70% are considered, responders lived significantly longer than nonresponders (median survival, 42 vs 23 weeks; $P = 0.05$). Patients presenting with a low initial PS (< 70%) did poorly with respect to

TABLE 2.—Results of treatment with FEMi

Characteristic	No. of patients with— Minor response	PR	CR	No. of patients responding/ No. in group (%)	*P* value*
Treatment with FEMi	12	10	3	25/63 (40)	—
Adenocarcinoma	7	6	2	15/40 (38)	NS
Large cell carcinoma	5	4	1	10/23 (43)	NS
PS					
< 70%	2	1	0	3/22 (14)	< 0.005
≥ 70%	10	9	3	22/41 (54)	
Prior weight loss					
> 10%	7	2	1	10/23 (43)	NS
< 10%	1	1	1	3/12 (25)	NS
None	4	2	1	7/16 (44)	NS
Unknown	0	5	0	5/12 (42)	NS

*NS = not significant.

survival duration regardless of response (median survival: responders, 6 weeks; nonresponders, 11 weeks; $P = 0.15$). The histologic subtype did not influence survival. Patients with adenocarcinoma lived a median of 23 weeks and those with large cell undifferentiated carcinoma lived a median of 19 weeks ($P = 0.35$).

In general, treatment with FEMi was well-tolerated. Eighteen patients (29%) did not experience any noticeable toxic reactions. Only five patients (8%) experienced vomiting. Severe myelosuppression was infrequent and necessitated a dose-rate reduction in only five patients. There were two episodes of life-threatening thrombocytopenia (platelets < 25,000/mm^3) and one patient with leukopenia died from pneumococcal sepsis. Other toxic effects noted were phlebitis (seven patients), mild to moderate alopecia (ten), mucositis (two), peripheral neuropathy (seven), jaw pain (two), and significant constipation (one).

DISCUSSION

Treatment of patients with metastatic adenocarcinoma and large cell undifferentiated carcinoma of the lung with FEMi resulted in an objective response rate of 40% and an overall median survival of 23 weeks. Patients having an objective response to FEMi had a median survival of 38 weeks. The pretreatment PS had a significant effect on both the response rate and survival time. Patients having an initial Karnofsky PS ≥ 70% had a 54% response rate and a median survival of 42 weeks for responders. Weight loss prior to starting therapy or after initiation of therapy did not influence the response rate or survival time. FEMi was well-tolerated by patients.

The substitution of vindesine for vincristine in the FOMi regimen did not result in any major improvement for FEMi-treated patients. The two treatment groups were very similar with regard to histology, extent of disease, and initial PS. A comparison of patients having an initial PS ≥ 70% and stage III M_1 disease indicates FOMi-treated patients had a 49% objective response rate and a median survival time for responding patients of 35 weeks while FEMi-treated patients had a 52% response rate and a 42-week median survival time. These differences are not statistically significant. The toxicity and side effects of the FOMi and FEMi treatment programs appear to be equivalent.

A major problem of the FEMi combination is the limiting effect of myelosuppression on the dose-rate schedule. The cumulative bone marrow toxicity of mitomycin limits the dose-rate schedule of vindesine to 25%–33% of that used in phase II trials of vindesine. Because of this necessary dose-rate reduction, the activity of the FEMi combination may be no better than any of the individual drugs given as single agents in a maximal dose-rate schedule. Methods of delivering a higher dose rate with acceptable toxicity need to be discovered, or active nonmyelosuppressive drugs need to be identified.

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Phase II Trial of Razoxane (ICRF-159) in Patients With Squamous Cell Carcinoma of the Head and Neck Previously Exposed to Systemic Chemotherapy[1,2]

Brief Reports

M. K. Shah,[3,*] P. F. Engstrom,[3,4] R. B. Catalano,[3,5] A. R. Paul,[3,6] R. E. Bellet,[3,7,8] and R. H. Creech[3,4]

Razoxane (ICRF-159), a nonpolar derivative of ethylenediaminetetraacetic acid was first synthesized at The Imperial Cancer Research Fund facilities in London, England. In preclinical studies it has been found to be active against L1210 leukemia (1), to inhibit pulmonary metastases from Lewis lung carcinoma (2), and to have antitumor synergism with irradiation in the Sarcoma 180 system (3). Synergism with doxorubicin, daunorubicin, and cisplatin has been reported (1,4). In a clinical phase I trial, a maximum tolerated dose of orally administered ICRF-159 was 3 g/m² in divided doses every 8 hours over 3 days, repeated every 3 weeks (5). A phase II trial of ICRF-159, conducted in patients with head and neck squamous cell carcinoma, is reported.

MATERIALS AND METHODS

Twenty-three patients with advanced squamous cell carcinoma arising in the head and neck and refractory to surgery, radiotherapy, and prior conventional chemotherapy were entered in this trial. Criteria for entry were measurable recurrent or metastatic disease and the following laboratory values: hemoglobin ≥ 9 g/100 ml, wbc count ≥ 4000/mm³, platelet count ≥ 100,000/mm³, total bilirubin ≤ 2 mg/100 ml, SGOT ≤ 80 IU/liter, and BUN ≤ 20 mg/100 ml. The patient population was composed of 17 men with a mean age of 63.5 years (range, 34–85) and six women with a mean age of 65 years (range, 60–72). Of the 23 patients, 11 had local disease and 12 had distant metastases; Eastern Cooperative Oncology Group (ECOG) performance status ranged from 0 to 3. Of the two responders, one had distant disease and a performance status of 3, and the other had regional disease and a performance status of 0. Seventeen patients had had prior surgery. All 21 evaluable patients had received radiotherapy and chemotherapy. Among the 21 patients, the following chemotherapeutic agents had been used either alone or in combination: methotrexate, all patients (100%); cyclophosphamide, two (10%); bleomycin, four (20%); doxorubicin, two (10%); 5-FU, one (5%); and cisplatin, one (5%). Five of 21 patients (24%) had partial responses to methotrexate; one had a partial response to bleomycin; and none responded to cyclophosphamide, doxorubicin, 5-FU, or cisplatin.

After giving written informed consent, each patient was treated with ICRF-159 at a dose of 800 mg/m²/day orally in three divided doses for 3 consecutive days. This therapy was repeated at 21-day intervals until evidence of progressive disease was noted. Patients who had wbc counts ≤ 3000/mm³ or platelet counts ≤ 100,000/mm³ on the day of therapy were not re-treated until hematologic values returned to normal. All patients were evaluated for response and drug toxicity at the initiation of each course of therapy. Oral, dermal, subcutaneous, nodal, and pulmonary lesions were measured in two dimensions. Determinations of serum SGOT, LDH, alkaline phosphatase, BUN, calcium, and glucose were performed at the initiation of therapy and at 6-week intervals. Hemoglobin determinations and wbc and platelet counts were performed weekly.

Patients were considered to be evaluable for toxic

[1]Received May 14, 1979; revised Aug 26, 1981; accepted Sept 18, 1981.

[2]ICRF-159 was supplied by the Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute.

[3]Department of Medicine, American Oncologic Hospital, Fox Chase Cancer Center, Philadelphia, PA.

[4]Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia.

[5]Philadelphia College of Pharmacy and Science, PA.

[6]Department of Medicine, Jefferson Medical College, Philadelphia, PA.

[7]Department of Medicine, Temple University School of Medicine, Philadelphia, PA.

[8]*Present address:* 1245 Highland Ave, Abington, PA.

**Reprint requests to:* Mukund K. Shah, MD, Department of Medical Oncology, Fox Chase Cancer Center, 7400 Central Ave, Philadelphia, PA 19111.

effects if there was a minimum followup of 14 days after the initiation of systemic chemotherapy, and they were considered to be evaluable for response if they had completed two courses of therapy (42 days). There were three response categories: *complete regression*—the disappearance of all measurable lesions, resulting in no clinical evidence of disease for at least 1 month; *partial regression*—a ≥ 50% decrease in the sum of the products of the perpendicular diameters of measurable disease without evidence of progression elsewhere for at least 1 month; and *no response*—all other evaluable patients.

RESULTS

Of 23 patients treated with ICRF-159, 21 received two or more courses of therapy and were, therefore, evaluable for response. Two patients were lost to followup within 14 days of starting treatment.

Toxicity

Of the 21 patients evaluable for toxic effects, two experienced anorexia, seven had nausea, three experienced mild emesis, two had significant but moderate alopecia, and one had diarrhea. Two patients had leukopenia (≥ 2000 cells/mm^3). None of the patients had a platelet count ≤ 100,000/mm^3, and none showed evidence of sepsis or hemorrhagic diathesis. One patient had anemia (on the basis of bone marrow suppression), which necessitated transfusion of two units of packed rbcs. There was no evidence of hepatic, pulmonary, or cardiac toxic effects in any patient treated with ICRF-159. None of the patients developed hypocalcemia. There were no ICRF-159-related deaths.

Response

Partial responses were achieved in two of the 21 patients evaluable for response. There was a > 50% decrease in the size of a neck mass in one patient and of a tracheostomy stomal recurrence in the other. The durations of response were 129 and 43 days, respectively.

DISCUSSION

Various chemotherapeutic agents have been used in the treatment of squamous cell carcinoma of the head and neck, with a wide range of reported response rates: methotrexate, 33% (9); 5-FU, 15% (9); 6-mercaptopurine, 12% (6); mechlorethamine, 7.5% (6); cyclophosphamide, 36% (9); chlorambucil, 14.7% (6); procarbazine, 10% (6); doxorubicin, 23% (10); bleomycin, 21% (6); and cisplatin, 30% (11). In this study, two of 21 patients (9.5%) had partial responses. Even though the patients had had previous chemotherapy, the response rate is comparable to response rates obtained with other single agents. The 95% confidence limits for the observed response rate are between 1.17% and 30.38%.

The toxic effects of ICRF-159 in this study are similar to those reported in earlier studies (5,7). Approximately 10% of the patients developed hematologic toxic effects that were moderate and reversible; anorexia, nausea, vomiting, or diarrhea occurred in about 50% of the patients; and 10% had moderate alopecia.

We conclude that ICRF-159 has a low degree of antitumor activity in squamous cell carcinoma originating in the head and neck region.

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Third-Line Chemotherapy With Mitomycin, Vinblastine, and Carmustine (BCNU) in Refractory Breast Carcinoma: A Pilot Study [1]

Brief Reports

Andrea Veronesi,* Umberto Tirelli, Enzo Galligioni, Mauro G. Trovò, Diana Crivellari, M. Donatella Magri, Mario Roncadin, Salvatore Tumolo, and Eligio Grigoletto [2]

SUMMARY

Twenty-four patients with breast carcinoma refractory to successive hormonal treatment, CMF (cyclophosphamide, methotrexate, and 5-FU) or its modifications, and AV (doxorubicin and vincristine) were treated iv with 3 mg/m² of vinblastine, 4 mg/m² of mitomycin, and 50 mg/m² of carmustine (BCNU) administered simultaneously every 14 days. This regimen was of little benefit in the 22 evaluable patients, yielding a 9% response rate.

[Cancer Treat Rep 66:559-561, 1982]

A high proportion of patients with disseminated breast cancer respond to multiple-drug therapy. Doxorubicin-containing regimens and combinations of cyclophosphamide, methotrexate, and 5-FU (CMF) with or without vincristine and prednisone yield a 50%–80% response rate with a median duration of response varying approximately from 5 to 10 months (1). A major problem is secondary treatment after failure of first-line chemotherapy. The doxorubicin-vincristine (AV) combination has proved to be non-cross-resistant with CMF (2). Mitomycin, vinblastine (VLB), and carmustine (BCNU) have a certain degree of activity (38%, 20%, and 21% response rates, respectively) in advanced disease as single agents (1) and have been used in breast cancer refractory to various primary chemotherapeutic combinations (3–10). The optimal chemotherapeutic treatment in patients who relapse after AV and second-line therapy with CMF (or the reverse) and are resistant to hormonal manipulations has not been investigated to our knowledge. Therefore, we undertook a pilot study to evaluate the efficacy and toxicity of a combination of mitomycin, VLB, and BCNU in patients with advanced breast carcinoma refractory to hormonal treatment, CMF or its modifications, and AV.

PATIENTS AND METHODS

From September 1979 to December 1980, 24 consecutive women with histologically proven metastatic breast adenocarcinoma were entered in the study. Conditions of eligibility were age < 70 years; failure after successive CMF or its modifications, AV, and hormonal treatment consisting of oophorectomy for premenopausal patients and tamoxifen for postmenopausal patients; measurable lesions; Karnofsky performance status > 40; wbc counts ≥ 4000/mm³; and platelet counts ≥ 120,000/mm³. Treatment consisted of simultaneous VLB (3 mg/m²), mitomycin (4 mg/m²), and BCNU (50 mg/m²) given iv every 14 days until disease progression. In patients who had previous myelosuppressive radiotherapy or massive bone involvement, the dose of BCNU was reduced to 30 mg/m². Clinical examination was performed before each cycle of chemotherapy; pertinent laboratory, roentgenographic, and scintigraphic studies were performed at regular intervals. When wbc counts were between 3000 and 4000/mm³ and/or platelet counts were between 90,000 and 120,000/mm³, 50% of the calculated dose was administered; for lesser values, therapy was delayed until bone marrow recovery.

Response was evaluated according to the standard criteria outlined by Hayward et al (11) and expressed as complete response, partial response (PR), no change (stable disease), or disease progression. Median age of the 24 patients was 53.5 years (range, 33–70). All patients were postmenopausal (in nine, surgical menopause dated from < 2 years before onset of treatment). Median performance status was

[1]Received Feb 5, 1981; revised Sept 25, 1981; accepted Oct 8, 1981.

[2]Division of Radiotherapy and Medical Oncology, Ospedale Civile, Pordenone, Italy.

Reprint requests to: Dr. Andrea Veronesi, Division of Radiotherapy and Medical Oncology, Ospedale Civile, 33170 Pordenone, Italy.

70 (range, 50–90). The involved sites were bone (11 patients); skin (nine); lymph nodes (seven); lung (five); liver (five); and primary breast tumor, brain, diaphragm, choroid, and ovary (one each). Apart from the above-mentioned chemohormonotherapy, previous treatment included radiotherapy with large fields in seven patients, medroxyprogesterone acetate in six, and mitomycin and BCNU in one patient each. CMF modification included CMF plus vincristine in one patient and CF plus vincristine in six.

RESULTS

Of the 24 patients in the study, two were unevaluable: one because of loss to followup and one because of early death due to cerebral hemorrhage. In the 22 evaluable patients, 97 cycles of therapy were administered. Four patients are still receiving treatment. Two patients (9%) had PRs, nine (41%) had no change, while 11 (50%) had disease progression. Durations of PR (one patient with skin, bone, and liver metastases and one with bone metastases) were 16+ and 2 months, respectively. Median duration of stable disease was 2 months. As regards disease sites, PR or stable disease was observed in five of 11 cases of bone metastases, three of seven lymph node metastases, and one of one ovary and diaphragm metastases and primary breast tumor. No response was observed in brain and choroid metastases. Median survival in the 11 patients with PR or no change was 4 months, while in the 11 with disease progression it was 3 months. The subjective toxicity encountered was significant. Almost all patients experienced nausea and vomiting, which was severe in 11. Five patients had malaise and nine had leukopenia and thrombocytopenia. It was possible to administer a median 95% of the theoretic dose of mitomycin, 96% of VLB, and 91% of BCNU. In the 97 cycles, therapy had to be delayed 22 times. No drug-related death was encountered.

DISCUSSION

The optimal medical treatment of patients with breast carcinoma resistant to conventional chemotherapy and hormone treatment has gained increasing attention in recent years, and several reports have dealt with this problem. AV can induce a 35% response rate in CMF failures (2). The results obtained in some recent studies with mitomycin, VLB, and BCNU alone or in combination in advanced refractory breast cancer are summarized in table 1. Continuous infusion of VLB appears to be the most active regimen in heavily pretreated patients.

The presence in the VATH and ABC combinations of a highly active drug such as doxorubicin makes it difficult to evaluate the relative importance of VLB and BCNU. Our study differs from the studies reported in table 1 in that all of our patients

TABLE 1.—Efficacy of second-line chemotherapy for advanced breast cancer in recent studies

No. of evaluable patients	No. of patients given previous hormonal treatment	Previous chemotherapy*	Second-line chemotherapy		Reference No.
			Drugs employed*	Objective response (%)	
14	10	CF, CAF, CAFM, CAFMV	VLB, MMC	7	8
20	18	AV and/or CMF	MMC	5	10
53	Not stated	CAFV	MMC, M, VM-26	30	3
30	Not stated	Combinations of C, A, M, F, HMM, MMC	VLB	40	7
19	Not stated	CMF, CMF→A, CMFVP, CMFVP→A, CAFVP	VLB, A, T, HAL (VATH)	52	5
48	30	Combinations of C, A, M, F, V	MMC, MA	27	6
27	9	CMF, CMFV, C, F	A, BCNU, C (ABC)	44	4
31	Not stated	CAMFVP, CAFVP	VLB, 6-TG	6	9
22	All patients	AV→CMF or CMF→AV in all patients. MMC and BCNU in 1 patient each	MMC, VLB, BCNU	9	Present study

*A = doxorubicin; C = cyclophosphamide; F = 5-FU; HAL = Halotestin; HMM = hexamethylmelamine; M = methotrexate; MA = megestrol acetate; MMC = mitomycin; P = prednisone; T = thiotepa; 6-TG = 6-thioguanine; V = vincristine.

were resistant to two successive non-cross-resistant drug combinations (AV and CMF) and to hormonal manipulations; therefore, they constitute a homogeneous population with a very poor prognosis. This can partly explain our results, which are not as encouraging as some of the data reported in the literature. In particular, our 9% PR rate compares unfavorably with the results of Buzdar et al (6) who used mitomycin in combination with a progestative agent. In that study, larger doses of mitomycin with longer rest intervals than ours were employed. Our results are instead comparable to those obtained by De Lena et al (10), with mitomycin alone administered in large intermittent doses, and those of Denefrio et al (8), with mitomycin and VLB given in a combination very similar to ours, but without BCNU. VLB and BCNU do not seem, therefore, to add any therapeutic efficacy to that of mitomycin alone. The association of mitomycin with VM-26 and methotrexate, as used by Pouillart et al (3), was far more efficacious than the drug combination employed in the present study. Again, mitomycin was administered at a higher dose than that given to our patients. Also, VLB used alone in a 5-day infusion was more efficacious (7) than the mitomycin, VLB, and BCNU in our series.

The poor therapeutic response we encountered was accompanied by a remarkable subjective toxicity: most patients had more bothersome nausea and vomiting or malaise than they had had with the previous conventional chemotherapy (AV and CMF). Bone marrow toxicity was not particularly significant.

In conclusion, it seems to us that the association of mitomycin with other myelotoxic drugs such as VLB and BCNU has little to offer to patients with refractory breast cancer, probably because of the reduction in dose of mitomycin. It is possible that the use of higher doses of mitomycin than we employed, in combination with less myelosuppressive agents than BCNU and VLB, could improve the results observed with mitomycin alone and those obtained in the present work.

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5-Azacitidine and Zorubicin for Patients With Previously Treated Acute Nonlymphocytic Leukemia: A Cancer and Leukemia Group B Pilot Study[1,2]

Brief Reports

Bruce A. Peterson,[3,*] Clara D. Bloomfield,[3] Arlan J. Gottlieb,[4] Morton Coleman,[5] and Mortimer S. Greenberg[6]

SUMMARY

Twenty-nine adults with advanced acute nonlymphocytic leukemia were treated with an intensive chemotherapy program consisting of 5-azacitidine and zorubicin. Overall, eight patients (28%) achieved a complete remission. Although only one of 19 patients with refractory leukemia responded, seven of ten patients who were treated immediately following relapse from a prior remission achieved another complete remission. The duration of remission ranged from 5 to 33 weeks (median, 14). Treatment resulted in severe toxic reactions including myelosuppression, renal tubular dysfunction, and elevations in liver enzymes. In addition, each of the 15 patients who died of treatment-associated complications had a severe infection. This combination of 5-azacitidine and zorubicin is relatively ineffective for truly refractory leukemia, but it may be a useful alternative following relapse.

[Cancer Treat Rep 66:563–566, 1982]

The results of further treatment in adults with acute nonlymphocytic leukemia who fail to respond to therapy with proven regimens or who have relapsed following an initial remission are largely unsatisfactory. Although progress has been made in the chemotherapeutic management of newly diagnosed patients, these advances have not greatly benefited patients who have previously received treatment. In recent studies, reinduction chemotherapy has often resulted in reductions in the peripheral leukocyte count and bone marrow cellularity, but complete remissions, particularly in patients with refractory leukemia, have occurred infrequently. Two new investigational agents active in the treatment of acute leukemia are 5-azacitidine (1–3), an antimetabolite, and zorubicin (4,5), an anthracycline antibiotic. Since these two agents with different mechanisms of action are active alone, a pilot study of their use in combination was undertaken.

PATIENTS AND METHODS

Twenty-nine adults with previously treated acute nonlymphocytic leukemia, including 17 males and 12 females ranging in age from 27 to 72 years (median, 54), were treated with 5-azacitidine and zorubicin. Three patients had developed leukemia following cytotoxic treatment for pre-existent malignancies. The median interval from the original diagnosis to treatment with 5-azacitidine and zorubicin was 11 months (range, 2–51). At the time of treatment with 5-azacitidine and zorubicin, the median hemoglobin was 10.3 g/dl, the median leukocyte count was 7.7×10^9/liter, the median platelet count was 47.0×10^9/liter, and the median myeloblast percent in the bone marrow was 55%. Ten patients had fever and six others had documented infections. Eight patients had abnormalities of liver function tests and three had elevated serum creatinine.

All of the patients had received prior intensive induction chemotherapy including, at least, cytarabine and either doxorubicin or daunorubicin. The cumulative amount of anthracycline previously administered ranged from 60 to 240 mg/m^2 (median, 150) of doxorubicin and 90–270 mg/m^2 (median, 135)

[1]Received Feb 25, 1981; revised July 31, 1981; accepted Aug 13, 1981.

[2]Supported in part by Public Health Service grants CA-16450, CA-21060, CA-07968, and CA-12449 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]University of Minnesota Medical School and Masonic Cancer Center, Minneapolis.

[4]State University of New York, Upstate Medical Center, Syracuse.

[5]New York Hospital-Cornell University Medical Center, New York.

[6]Mt. Auburn Hospital, Cambridge, MA.

**Reprint requests to:* Bruce A. Peterson, MD, Box 348, University Hospitals, University of Minnesota, 420 Delaware St, SE, Minneapolis, MN 55455.

of daunorubicin. Thirteen of the patients had also received additional drugs, most commonly 6-thioguanine. Initial induction treatment had resulted in complete remissions for 16 patients. Following relapse, nine of these 16 patients had reinduction attempts with standard agents and three obtained a second complete remission. Thus, the six patients who failed to obtain second remissions and 13 others who had never achieved an initial remission were refractory to standard agents.

The induction chemotherapy regimen consisted of 5-azacitidine on Days 1–5 and zorubicin on Days 1–3. One fourth of the daily dose of 5-azacitidine (50 mg/m^2), freshly reconstituted in lactated Ringer's solution, was administered over alternating 3-hour intervals, for a total dose of 200 mg/m^2/day. Zorubicin (175 mg/m^2/day) was administered on Days 1–3 as an iv infusion over 1 hour following premedication with diphenhydramine hydrochloride (Benadryl) at a dose of 50 mg iv. A second course of therapy, when required, was started ≥ 14 days after the initiation of the first course and consisted of 5-azacitidine for 5 days and zorubicin on Days 1 and 2. Bone marrow aspiration and biopsy were repeated on the 14th day and, subsequently, at weekly intervals. All patients who achieved a complete remission were placed on maintenance chemotherapy.

The patients' remission status following therapy was assessed using the criteria of the Cancer and Leukemia Group B (6). The duration of remission was measured from the date a complete response was documented to the date of hematologic relapse, when either the bone marrow myeloblast percent increased and was maintained > 5% or leukemic forms reappeared in the blood or marrow. Survival was calculated from the date chemotherapy with 5-azacitidine and zorubicin was begun.

RESULTS

Response

Eight (28%) of the 29 patients undergoing induction treatment with 5-azacitidine and zorubicin achieved a complete remission. One patient who showed a good initial response to treatment refused a second course of therapy. An additional patient appeared to be entering complete remission when she died from fungemia secondary to *Candida albicans*. Seven patients achieved complete remission with only one course of chemotherapy; one required two courses. The time from initiation of treatment to complete remission was relatively long, ranging from 31 to 78 days (median, 50).

No differences were observed in pretreatment hematologic characteristics or in the age and sex of the responders and nonresponders. One of the three patients who developed leukemia following cytotoxic chemotherapy for a prior malignancy achieved complete remission. All but one of the patients who entered remission had had a previous complete remission. That patient was the only responder who had been clearly refractory to other drugs before responding to 5-azacitidine and zorubicin. Thus, seven of ten patients treated immediately following relapse achieved remission compared to only one of 19 patients who were refractory to standard agents ($P < 0.005$).

The median duration of complete remission was 14 weeks (range, 5–33). Six patients received maintenance chemotherapy consisting of monthly courses of 5-azacitidine in combination with either zorubicin, cyclophosphamide, or 6-thioguanine rotated in sequence. This program produced prolonged myelosuppression. Two patients received oral 6-mercaptopurine at a dose of 50 mg/m^2/day and weekly oral methotrexate at a dose of 15 mg/m^2/week.

Toxicity

Toxicity associated with the administration of this treatment regimen was severe. Nausea and/or vomiting were seen in 16 patients and diarrhea was reported in four patients. Profound leukopenia developed in 24 of the 27 patients for whom data were available. Two patients died on Day 9 while their wbc counts were still decreasing and a third patient's leukocyte count never decreased to < 1.3 × 10^9/liter. In the other 24 patients, the leukocyte counts decreased to < 1.0 × 10^9/liter within 2–11 days (median, 6) after initiating chemotherapy and remained < 1.0 × 10^9/liter from 5 to 43 days (median, 20). The median nadir leukocyte count was 0.1 × 10^9/liter and the median platelet count was 8.0 × 10^9/liter. Sixteen of the 19 patients whose bone marrow examinations were repeated following treatment showed some evidence of response by developing hypocellularity and substantial reductions in their myeloblast percents.

All patients had fevers during the induction period, and 20 had additional evidence of significant infection. Seven patients developed pulmonary infiltrates and 13 had documented generalized sepsis. Seven patients developed hemorrhagic complications. Fifteen patients had hepatic function abnormalities usually manifested by an elevation of the serum alkaline phosphatase and the SGOT to approximately twice normal. Five patients became jaundiced and hepatic failure was thought to play a

role in the death of one patient. Five patients developed cardiac abnormalities following one complete course of treatment. Atrial or ventricular arrhythmias were present in three patients and congestive heart failure was present in two. One of these patients had previously received 240 mg/m^2 of doxorubicin and the others had received 135–270 mg/m^2 of daunorubicin.

Ten of 13 patients who were serially evaluated for renal tubular dysfunction developed abnormalities consistent with tubular defects. Seven patients had polyuria (maximal urine output, 4.5–39.9 liters/day) associated with weight loss and orthostatic hypotension. Nine patients had an alkaline urine in the presence of a low serum bicarbonate (range, 11–23 mEq/liter). Eight patients developed hypophosphatemia with values for serum phosphorus ranging from 1.0 to 1.9 mg/dl and three patients developed glucosuria while serum glucose values remained < 150 mg/dl. All but three of the patients with renal tubular dysfunction also had mild elevations in serum creatinine of 1.5–2.7 mg/dl. These manifestations were transient, beginning within a few days of chemotherapy and ending 7–10 days later.

Survival

The median survival for all patients included in this study was only 52 days. Thirteen of the patients (45%) survived for ≤ 4 weeks after the initiation of chemotherapy and 15 died from complications associated with their treatment. All of these patients died with severe infections and in two patients congestive heart failure and hepatic failure, respectively, were thought to be contributing factors. One patient died while still in complete remission. The median survival of the patients entering complete remission was 32 weeks.

DISCUSSION

Therapy remains a difficult problem for patients with acute nonlymphocytic leukemia who have relapsed following a remission or are refractory to initial induction attempts. Despite the reduction in leukemic cell mass that can be obtained with a variety of drugs or drug combinations, complete remissions occur infrequently and when achieved, are generally brief. 5-Azacitidine and zorubicin are effective new agents. When used alone, 5-azacitidine results in a complete remission rate of approximately 20% in patients with previously treated leukemia (1–3). 5-Azacitidine has also been tested in combination with a variety of other drugs where complete response rates have ranged up to 29% in small groups of previously treated adults; however, in patients proven refractory to other agents, responses are uncommon (7–10). Marked activity has been claimed for zorubicin in acute nonlymphocytic leukemia. Response rates as high as 33% in previously treated patients (4) and 57% in previously untreated patients (5) have been reported. The complete response rate of 28% observed in the present study for the combination of 5-azacitidine and zorubicin is not substantially different from rates previously reported in previously treated patients for either agent alone. However, seven of the ten patients who had previously responded to standard agents and who were treated immediately following relapse from either a first or second complete remission obtained another remission, compared to only one of 19 patients who had the refractory nature of their leukemia established before receiving 5-azacitidine and zorubicin. Thus, although 5-azacitidine and zorubicin together are relatively ineffective for truly refractory disease, they can be effective following relapse.

The toxicity associated with this treatment program was considerable. Prolonged and severe myelosuppression occurred in nearly every patient, and infection contributed to the deaths of all who died of treatment-associated complications. The number of deaths attributed to infection, despite vigorous supportive care, and the occurrence of hemorrhagic complications in seven patients underscore the serious myelotoxicity observed. Zorubicin has been reported to be less cardiotoxic than either daunorubicin or doxorubicin (5,6). In the present study, five patients developed cardiac abnormalities following single courses of treatment. However, because of the complexity of the clinical situation, it is difficult to attribute these events solely to the cumulative cardiotoxic effects of current or previous chemotherapy. It is now apparent that an array of abnormalities characteristic of diffuse renal tubular damage may be associated with the administration of 5-azacitidine in combination with other agents (11), and this was observed again in the present study. Because the occurrence and the severity of renal dysfunction are not predictable, all patients receiving 5-azacitidine should be monitored closely.

5-Azacitidine and zorubicin are both active agents in patients with advanced acute nonlymphocytic leukemia. In relapsed patients, this combination may be a useful alternative, and in newly diagnosed patients, these drugs may become useful adjuncts to current treatment programs. However, for patients with truly refractory leukemia, more effective drugs and newer approaches will be necessary to obtain even initial complete responses.

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Phase II Study of the Combination of Vinblastine, Bleomycin, and Cisplatin in Advanced Malignant Melanoma[1]

Brief Reports

Edward T. Creagan,* David L. Ahmann, Allan J. Schutt, and Stephanie J. Green[2]

SUMMARY

Eighteen patients with disseminated malignant melanoma received a monthly iv regimen of vinblastine, bleomycin, and cisplatin; 17 patients had had no prior chemotherapy, and 12 had predominantly visceral disease. An 11% response rate concomitant with substantial gastrointestinal and hematologic toxic effects was achieved. We conclude that in the dose and schedule used, the vinblastine, bleomycin, and cisplatin regimen had minimal activity against malignant melanoma.

[Cancer Treat Rep 66:567–569, 1981]

Selected polychemotherapy for advanced malignant melanoma achieves a response rate of approximately 15% (1–3). Therefore, we were especially interested in the combination of vinblastine, bleomycin, and cisplatin (VBP), which produced ten (71%) regressions among 14 patients (4). The analysis excluded eight additional patients from evaluation because of limited followup (six) or death within 7 days of starting treatment (two). We report here a phase II study of the VBP regimen in patients at the Mayo Clinic with disseminated malignant melanoma.

MATERIALS AND METHODS

Eighteen patients (nine males and nine females; median age, 54 years) with biopsy-proven, assessable (13 measurable and five evaluable), advanced melanoma participated in the study. Twelve patients had predominantly visceral disease, and six had predominantly soft tissue involvement. The Eastern Cooperative Oncology Group (ECOG) performance scores were 0 and 1 in 14 patients and 2 and 3 in four patients. Seventeen patients had had no prior systemic chemotherapy; three with brain metastases had received prior radiation therapy.

The initial iv doses repeated at 4-week intervals were: vinblastine, 6 mg/m² on Days 1 and 2; bleomycin, 15 units/m²/24 hours on Days 1–5; and cisplatin, 50 mg/m² on Day 5. Cisplatin was administered at the end of a 4-hour infusion of 2000 ml of 5% dextrose in 0.45 normal saline, with 50 mg of furosemide given iv at the start of the cisplatin infusion. After the cisplatin infusion, urine output of 200–300 ml/hour was maintained for at least 6 hours.

EVALUATION

Prior to initial treatment and subsequent monthly courses, patients had a complete medical evaluation including chest X-ray and routine hematologic and chemical tests.

Objective response was defined as a decrease of > 50% in the product of the longest perpendicular diameters of the most clearly measurable indicator lesion, in the absence of symptomatic deterioration. Progressive disease was defined as a > 25% increase in the product of the perpendicular diameters of any indicator lesion or the appearance of new lesions. Time to treatment failure was calculated from Day 1 of treatment to the date of disease progression or death.

RESULTS

Response

We achieved two objective regressions (11%) among 18 patients. A 50-year-old man had a > 50% decrease in a clearly measurable lung lesion for 5 months, and a 53-year-old man continues (4+ months) to have complete regression of a chest wall mass that had recurred after biopsy confirmation. Neither patient had had prior chemotherapy. Of the 18 patients, 39% were immediate treatment failures.

[1]Received June 5, 1981; revised Aug 24, 1981; accepted Sept 18, 1981.

[2]Division of Medical Oncology (*E. T. Creagan, D. L. Ahmann,* and *A. J. Schutt*) and Cancer Statistics Unit (*S. J. Green*), Mayo Clinic, Rochester, MN.

**Reprint requests to*: E. T. Creagan, MD, Medical Oncology, Mayo Clinic, Rochester, MN 55905.

TABLE 1.—Toxic effects and response data in 18 patients

Drug (mg/m²)			Patient characteristics				Hematologic toxicity*	
Vinblastine (Days 1,2)	Bleomycin (Days 1–5)	Cisplatin (Day 5)	No. of patients	Performance score	Prior systemic chemotherapy	No. of regressions	Wbc count nadir < 3.0 cells × 10^3/mm³	Platelet count nadir < 130 cells × 10^3/mm³
6	15	50	2	0,2	No	0	0.6 (6), 3 (7)	45 (3)
4	15	50	1	1	No	0	0.8 (7)	37 (2)
3	8	30	3	1,1,1	No	0	No toxicity	No toxicity
4	12	45	5	0,0,1,2,2	Yes†	1	1.7 (15),‡ 1.0 (7), 1.7 (11)‡	73 (5),‡ 51 (9)
6	15	50	3	0,0,1	No	1	0.4 (8), 0.2 (8),§ 2.5 (14)‡	111 (4), 100 (35)‡
4	15	50	4¶	0,1,1,2	No	0	2.6 (4), 2.6 (11), 2.7 (5)	28 (3), 68 (4)

*Values in parentheses represent Day of treatment on which nadir occurred.
†1 patient had received DTIC, BCNU, and PALA.
‡Nadir occurred later than 1st cycle.
§Patient died from sepsis 10 days after start of treatment.
¶Increase in serum creatinine and enlarged kidneys in 1 patient and pulmonary toxicity in 1.

The median (Kaplan-Meier) times to failure and survival were 2 and 7 months, respectively.

Toxicity

The first three patients developed significant hematologic toxic effects and substantial nausea and vomiting within 7 days of starting treatment (table 1). To more precisely define hematologic toxic effects, eight additional patients received lower doses of each agent. Because it appeared that the initial doses could be safely administered (table 1), the study was resumed at these doses in three additional patients. One of these patients had a wbc count of 200/mm^3 8 days after starting treatment and died from sepsis 2 days later, and another had a wbc count of 400/mm^3 8 days after starting treatment but recovered without sequelae. Four other patients received a somewhat reduced dose of vinblastine; three had only mild leukopenia, but a platelet count of 28,000/mm^3 occurred on Day 3 in one.

Forty-eight hours after receiving vinblastine and bleomycin, but prior to receiving cisplatin, a 26-year-old patient developed gross hematuria, with an increase in serum creatinine from 1.1 to 3.2 mg/dl over 6 days. An excretory urogram and an abdominal computerized tomographic scan showed diffusely enlarged kidneys (right, 17 cm; left, 18.6) but no evidence of obstructive uropathy. Cystoscopy identified acute, diffuse hemorrhagic cystitis. The serum creatinine remains increased at 1.7 mg/dl 8 weeks after the initial insult. It is unclear whether the event was related to the disease or to the treatment.

A 66-year-old female patient, with a performance score of 1, had clinical and roentgenographic evidence of bleomycin-induced pulmonary toxic effects after a total dose of 184 units.

All patients had some nausea and vomiting, which was moderate to severe in 61%. Other toxic effects included alopecia (61%), stomatitis (33%), constipation (22%), diarrhea (17%), and fever (6%).

DISCUSSION

Despite a sound theoretic background for the VBP regimen (5) and promising preliminary studies (4), we were unable to achieve meaningful clinical benefit among 18 patients with advanced melanoma, who experienced severe toxic effects. We clearly recognize that subtle clinical, chemical, or immunologic differences among reported study populations and the influence of unrecognized covariates might account, in part, for response discrepancies, but this seems doubtful in our view. The overall results are inferior to most published studies of combination chemotherapy for advanced melanoma, and the toxicity would obviate routine clinical use of the reported VBP regimen. We were especially concerned about lethal complications following modest increases in drug dose and about a very narrow therapeutic index.

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Cerebrospinal Fluid Versus Serum Concentrations of 5-FU, Allopurinol, and Oxypurinol During Treatment of Metastatic Brain Cancer With 5-FU Infusion, Allopurinol, and Radiation[1]

Brief Reports

C. L. Hornbeck,[2,*] R. A. Floyd,[2] J. E. Byfield,[3] J. C. Griffiths,[4] S. Frankel,[3] and T. R. Sharp[5]

SUMMARY

Concurrent cerebrospinal fluid (CSF) and serum concentrations of 5-FU, allopurinol, and its metabolite, oxypurinol, were measured in three patients with metastatic brain cancer who had been treated with 5-FU infusion, oral allopurinol, and whole-brain radiation. Little or no 5-FU was found in the CSF despite therapeutic 5-FU serum concentrations, but significant concentrations of oxypurinol were detected in the CSF.

[Cancer Treat Rep 66:571–573, 1982]

Previous reports have indicated that after bolus doses of 5-FU, the cerebrospinal fluid (CSF) of man and other primates contained 5-FU (1–3). In addition, there has been evidence of CNS toxicity in recent trials of both 5-FU and allopurinol (AP) in patients with non-CNS tumors (4,5). Recent studies have indicated that concurrent 5-FU and AP administration affords some systemic protection from 5-FU toxicity (6,7); however, AP may not provide this same protective effect for the CNS. It is still unclear whether concomitant AP produces a significant increase in the therapeutic ratio between tumor and normal tissue (8).

Radiation therapy and 5-FU have been found to be synergistic in killing human tumor cells in vitro (9). Subsequent clinical trials have shown increased response rates in esophageal cancer when 5-FU was infused for 120 hours to maintain systemic concentrations of approximately 400 ng/ml in combination with daily radiation therapy (10). These findings suggested that concomitant 5-FU and AP treatment could increase the response of intracranial tumors to radiation therapy by increasing delivery of 5-FU to brain tumor cells without increasing systemic toxicity. We report concurrent CSF and serum concentrations of 5-FU, AP, and oxypurinol (OP) found in three patients with metastatic brain cancer who were treated with 5-FU, AP, and radiation.

MATERIALS AND METHODS

Three male patients with metastatic brain cancer received continuous infusion of 5-FU (28 or 30 mg/kg) and whole-brain irradiation (400 rads) daily for 5 days. Two of these patients received oral AP at a dose of 300 mg three times a day and the third received 100 mg three times a day. One of the patients receiving AP at the 300-mg three times a day dose was later treated with the same regimen but no AP. A fourth male patient, who had no brain metastasis, was treated with 35 mg/kg/day of 5-FU but no AP or whole-brain radiation. Four or 5 days into each cycle, a single lumbar CSF sample was taken simultaneously with venous blood from each patient for determination of 5-FU, AP, and OP concentrations. Blood samples were taken daily to monitor steady-state levels of 5-FU.

Serum and CSF concentrations of 5-FU, AP, and OP were assayed by reverse-phase, high-performance liquid chromatography sensitive to 10 ng/ml of 5-FU (11). The method was modified by attaching a Waters RCM-100 radial compression module containing a 5-mm inner diameter liquid chromatography cartridge (Radial-Pak C_{18}) to a Hewlett-Packard 1084B liquid chromatograph. Retention times

[1]Received July 28, 1981; revised Oct 2, 1981; accepted Oct 13, 1981.

[2]Departments of Laboratory Service (*C. L. Hornbeck*) and Pharmacy Service (*R. A. Floyd*), Veterans Administration Medical Center, San Diego, CA, and University of Southern California School of Pharmacy, Los Angeles.

[3]Department of Radiation Oncology, University of California Medical Center, San Diego.

[4]Department of Pathology, Sharp Memorial Hospital, San Diego, CA.

[5]Department of Radiation Oncology, Veterans Administration Medical Center, San Diego, CA.

**Reprint requests to:* Dr. Cecil L. Hornbeck, Department of Laboratory Service (113), Veterans Administration Medical Center, 3350 La Jolla Village Dr, San Diego, CA 92161.

TABLE 1.—CSF vs serum concentrations of 5-FU, AP, and OP from patients continuously infused with 5-FU with or without oral AP

Patient No.*	Total daily dose		Single simultaneous CSF/serum concentrations†			Daily serum 5-FU concentration‡ (ng/ml)
	5-FU (mg/kg)	AP (mg)	5-FU (ng/ml)	AP (μg/ml)	OP (μg/ml)	
1	28	300	ND/644	0.07/0.06	1.2/5.1	477 ± 191
2	30	900	ND/317	0.28/0.33	5.4/18	356 ± 52
3	28	900	ND/131	0.13/0.56	5.2/16	511 ± 63
3	28	0	ND/985	—	—	—
4	35	0	22/850	—	—	500 ± 440

*Patient 1 had primary right axillary tumor and brain metastasis; patients 2 and 3 had primary lung tumor and brain metastasis, and patient 3 was re-treated after 6 wks without AP; patient 4 had primary lung tumor, no brain metastasis or brain irradiation, and questionable CNS toxicity.

†ND = not detected.

‡Values = mean and SD of 3–5 serum samples taken during treatment cycle.

on this column were 1.9, 4.1, 4.8, and 13.4 minutes, respectively, for 5-FU, OP, AP, and the internal standard, thymidine.

RESULTS AND DISCUSSION

The total daily 5-FU and AP doses and the serum and CSF concentrations of 5-FU and the two xanthines are listed in table 1. Despite therapeutic serum 5-FU concentrations for up to 4 or 5 days, little or no 5-FU was found in the CSF regardless of AP administration. On the other hand, it appeared that most of the AP was metabolized to OP, which in turn was present in significant concentrations in the CSF. The CSF concentrations of OP were about 50% of the serum concentrations. After 4–5 days of AP administration, the serum xanthine levels should be at approximately steady-state concentrations.

The three patients with brain metastasis experienced mild toxic effects (no CNS toxicity); one was re-treated without AP but with the same 5-FU and radiation regimen and still experienced only mild toxic effects. A fourth patient, who had no brain metastasis or AP treatment, had mild ataxia with a higher daily dose of 5-FU (35 mg/kg).

Combined with previous findings in man, our results indicate that iv bolus doses of 5-FU and the accompanying initially high microgram serum concentrations of 5-FU are necessary to produce nanogram concentrations of 5-FU in the CSF. In a previous study, two patients had transitory concentrations of 5-FU that actually exceeded the serum 5-FU concentrations for several hours after an iv bolus dose of 15 mg/kg (2). This circumstance is most probably explained by the generally slower clearance of CSF as opposed to serum. In this same study, a single patient given 30 mg/kg/day of 5-FU by continuous infusion had no measurable CSF levels of 5-FU (2). In another study, primates given iv bolus doses of 5-FU that were injected more slowly had lower CSF concentrations of 5-FU than those given rapid iv bolus injections of the same total dose (1). It is apparent that under the conditions of this study the rapid systemic clearance of 5-FU does not allow significant penetration of the polar 5-FU molecule into the CSF with or without AP.

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Combination Chemotherapy for the 9L Rat Brain Tumor Model System[1,2]

Brief Reports

Tribhawan S. Vats,* Bruce F. Kimler, Sheri D. Henderson, and Robert A. Morantz[3,4]

SUMMARY

Utilizing the 9L intracerebral rat brain tumor system, we have demonstrated that the combination of bleomycin delivered ic and BCNU (carmustine) given systemically is more therapeutically effective than either agent used alone. This benefit can be achieved without excessive short-term normal tissue damage. Furthermore, the potential for long-term normal tissue effects is reduced because of the low dose of bleomycin required. There was no difference between bleomycin given 16 hrs before BCNU administration and the reverse sequence. These results suggest the possibility of adding intratumor bleomycin to protocols using BCNU for the treatment of malignant human brain tumors.

[Cancer Treat Rep 66:575–579, 1982]

The principal approach to the treatment of human brain tumors has been radiotherapy and surgery, but with little success since the probability of long-term survival remains low. In recent studies, chemotherapy has been used not only for treatment of recurrent disease but also as an adjuvant with radiation therapy for primary disease. The use of chemotherapy has been hampered by the existence of the blood-brain barrier, inability of certain drugs to attain adequate cerebrospinal fluid (CSF) levels, and dose-limiting systemic toxicity.

Two nitrosoureas that have been shown to be effective for brain tumor therapy are BCNU (carmustine) and CCNU (lomustine).[5] Their lipid solubility and adequate CSF penetration make them ideal agents when used alone or in combination schedules. The dose-limiting toxic effects are myelosuppression and pulmonary fibrosis (1). BCNU alone has been shown to improve survival in the same experimental rat brain tumor (9L gliosarcoma) model as used here (2), and its effectiveness is enhanced when combined with radiation therapy (3–5). In one laboratory, maximum enhancement was obtained when BCNU was given 16 hrs prior to radiation therapy (3,4).

Bleomycin is a glycopeptide obtained from *Streptomyces verticillus* that has shown activity against a wide variety of human neoplasms, but its transport to the brain is limited by the existence of the blood-brain barrier. In a previous study (6), we demonstrated the effectiveness of bleomycin when administered directly to the 9L tumor by an ic route. In the present report, we attempted to determine if the combination of intratumor bleomycin and systemically administered BCNU would improve the survival of rats bearing intracerebral 9L tumors compared to the survival of rats treated with these agents singly. The effect of schedule dependency was also investigated using BCNU 16 hrs before or after bleomycin therapy.

MATERIALS AND METHODS

The techniques for achieving reproducible intracerebral 9L tumors in our laboratory have been reported previously (7). A dose of 1 mg/kg was routinely used for the direct ic administration of bleomycin. Animals were lightly anesthetized with

[1]Received Apr 17, 1981; revised Aug 26, 1981; accepted Sept 8, 1981.

[2]Supported in part by Public Health Service grant CA-19118 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. S. D. Henderson was supported by a predoctoral fellowship from the American Association of University Women.

[3]Department of Pediatrics, Division of Oncology (*T. S. Vats*), Radiation Biology Laboratory, Department of Radiation Therapy (*B. F. Kimler* and *S. D. Henderson*), and Brain Tumor Research Laboratory, Department of Surgery (*R. A. Morantz*), University of Kansas Medical Center, Kansas City.

[4]We acknowledge the expert technical assistance of R. D. McDonald.

**Reprint requests to:* Dr. Tribhawan S. Vats, Department of Pediatrics, University of Kansas Medical Center, Rainbow Blvd at 39th St, Kansas City, KS 66103.

[5]Drugs were provided by Bristol Laboratories, Syracuse, NY.

the inhalant Metafane (Pittman-Moore, Inc, Washington Crossing, NJ), the original site of tumor inoculation was opened, and the drug was administered through a 25-gauge needle of an automatic syringe directly into the tumor area (precisely at 3.5 mm beneath the skull). With this technique, only 5 μl of the drug solution was delivered so as to retain all drug in the tumor volume. The technique provided adequate drug delivery with minimum acute morbidity. Those animals that died as a result of the procedure itself (usually < 10%) did so within a matter of hours due to comas, seizures, etc, and were excluded from the analysis. BCNU (100 mg) was dissolved in 3 ml of absolute ethanol and 27 ml of sterile saline. A dose of 12 or 13 mg/kg was administered ip within 10 mins of preparation. Data were analyzed using the endpoint of animal survival. Median survival times (MSTs) were calculated and tested for statistical significance using a Mann-Whitney test, and, where necessary, a Wilcoxon test for singly censored samples (8).

RESULTS

Figure 1 demonstrates the increase in MST of 9L tumor-burdened rats treated with bleomycin alone, BCNU alone, or a combination of the two agents. Based on our earlier studies (6), bleomycin was administered ic at a dose of 1.0 mg/kg weekly until death, starting on Day 7 after tumor inoculation. A BCNU dose of 12 mg/kg ip on Day 14 after inoculation was used. Compared to the control MST (25 days), the MSTs for bleomycin only (31 days) and BCNU only (37 days) were significantly better ($P < 0.05$). For multiple-drug therapy, the two treatment schedules were combined. The MST for the combination (40 days) was better ($P < 0.05$) than for the controls or bleomycin only, but only marginally more effective than BCNU alone ($P \approx 0.10$). In part, this was due to complications resulting from the weekly administration of bleomycin and the attendant anesthesia and minor surgical procedures involved. Animals that are well-advanced in the disease process are weakened as a result and do not tolerate the repeated procedure. They do not die immediately after or during the procedure, indicating that they are not dying from acute morbidity. However, over the next several days they seem to die sooner than would have been predicted from their status. For example, rather than taking the typical 4–6 days to die after first appearance of the effects of the brain tumor, animals die within 1–2 days with the time-course of symptoms accelerated. Thus, we do not consider this effect to be a toxic effect related to the bleomycin but rather to the procedure involved in the ic drug delivery. In an effort to reduce these complications, we have tested the use of single bleomycin treatments and have found that this is almost as effective as the repeated administrations, yet produces no toxicity problems other than the minimal and immediate acute morbidity. In the clinical situation where multiple administrations will certainly be required, a similar problem will not exist since bleomycin will be administered via an Ommaya reservoir, thus avoiding anesthesia and surgery.

In another experiment (fig 2), we treated tumor-burdened rats with a single bleomycin treatment, a single BCNU administration, or the combination of the two agents used in two different schedules. The MST of control animals (treated with 5 μl of phosphate-buffered saline ic) was 22 days. Prolongation of MST was observed with either bleomycin (28 days) or BCNU (38 days) alone, or with the combination treatments (47 and 49 days). These prolongations were all significant ($P < 0.001$). There was no difference in MST ($P > 0.98$) when BCNU was given 16 hrs before vs 16 hrs after bleomycin treatment, indicating a lack of the schedule dependency that had been demonstrated with BCNU and radiation (3,4). However, the increase in MST after combined treatment was significantly better than after treatment with bleomycin alone ($P < 0.001$) or BCNU alone ($P < 0.025$).

DISCUSSION

These results indicate that bleomycin can be effectively combined with BCNU for the treatment of an intracerebral rat brain tumor. We were able to combine both agents using doses close to the maximum tolerated levels for each single agent. Maximum prolongation of survival by bleomycin is produced at a dose of 1.0 mg/kg (6). Beyond that, MST decreases as more animals die of complications associated with acute bleomycin toxicity (6). Complications due to the repeated treatment procedure (resulting in animals dying several days early) can be avoided by using only a single bleomycin administration, with no loss of therapeutic effectiveness. Likewise, the most effective dose of BCNU in experimental studies has been 13.3 mg/kg ip (2). This mode of combination chemotherapy offers some distinctive advantages which would be lacking if both agents were given systemically. With the intratumor route, the concentration of bleomycin that is therapeutically effective is much less than the amount required by the systemic route. Bleomycin at the dose used does not cause myelosuppression and thus myelosuppression by BCNU will not be enhanced.

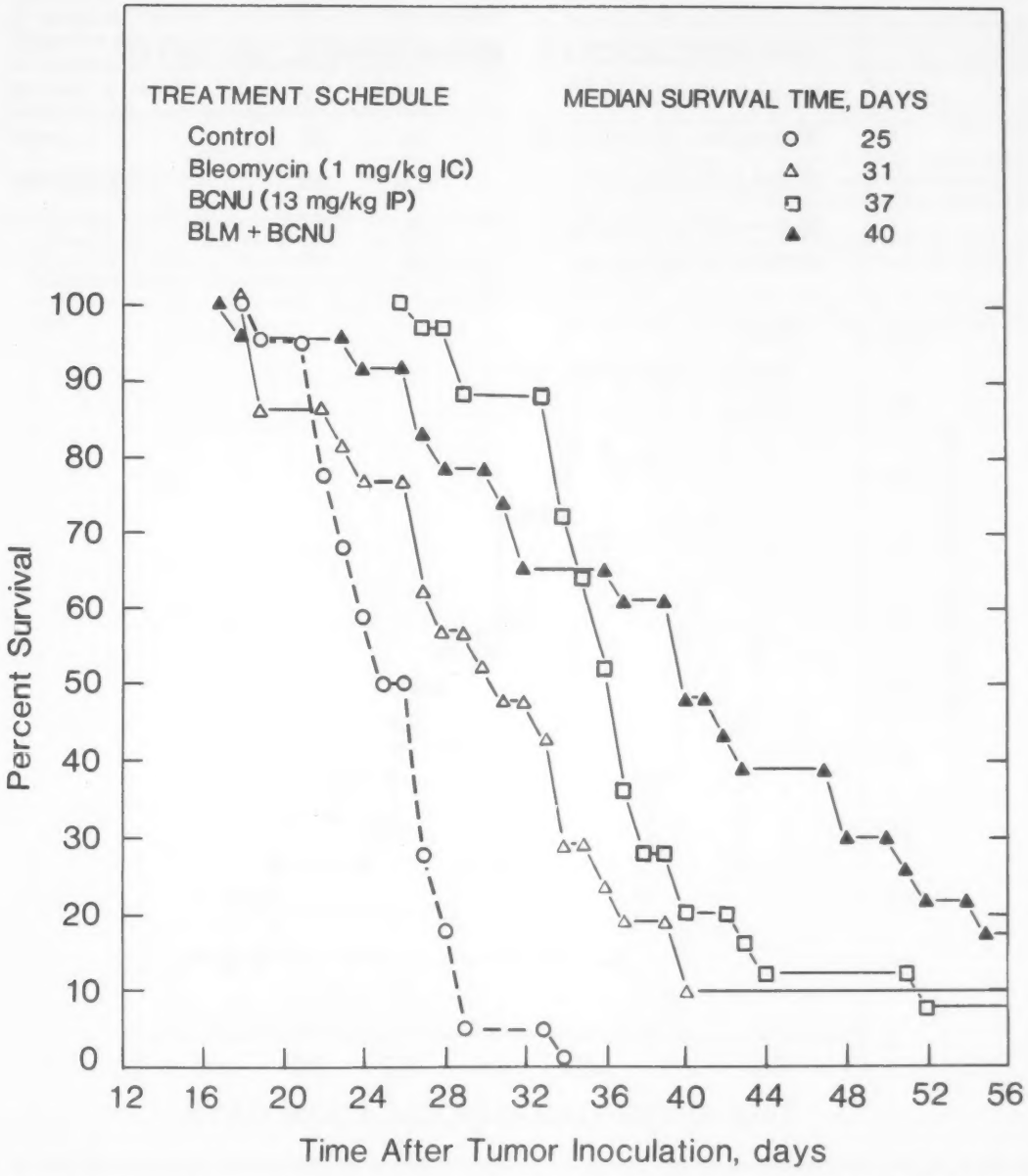

FIGURE 1.—Survival of 9L tumor-burdened rats. Bleomycin therapy was given weekly until death, starting on Day 7 after tumor inoculation, and BCNU therapy was given on Day 14 after tumor inoculation. MSTs are as listed.

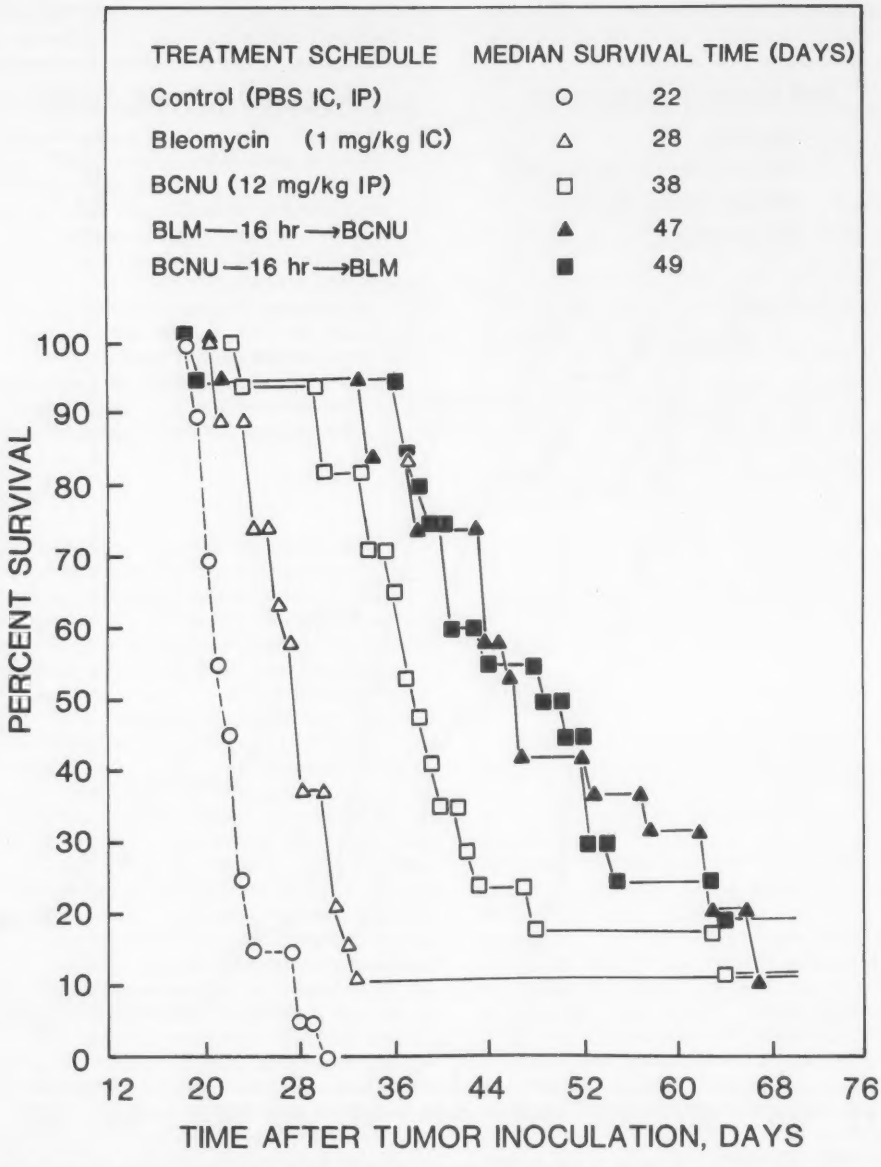

FIGURE 2.—Survival of 9L tumor-burdened rats. Therapy was performed on Days 10 and 11 after tumor inoculation. MSTs are as listed.

Additionally, the danger of potentiation of pulmonary fibrosis is reduced. In clinical situations where patients have received a maximum tolerated dose of radiation therapy and/or BCNU (and further treatment can not be administered safely), it may be possible to deliver intratumor bleomycin in an attempt to control disease and prolong survival without producing unacceptable normal tissue toxic effects.

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A Pilot Study of Estracyt in Advanced Breast Cancer[1]

Clinical Trials Summaries

P. J. D. K. Dawes[2,*]

A synergistic effect of combining endocrine therapy and an alkylating agent was observed by Watson and Turner (1). They suggested the synthesis of compounds incorporating an alkylating agent and a sterol hormone. Estracyt (estramustine phosphate) is one such drug. In Estracyt, mechlorethamine has been conjugated to estradiol 17-phosphate in position 3.

Estracyt was shown by Fredholm et al (2) to inhibit the growth of mammary carcinomas in animals. The inhibiting effect was thought to be mainly due to the cytotoxic activity of Estracyt.

There is also evidence that Estracyt is selectively concentrated in hormone-responsive target tissues (3).

A pilot study of Estracyt therapy for advanced breast cancer was undertaken. Such a study has previously been reported by Alexander et al (4). The European Organization for Research on Treatment of Cancer (EORTC) study (5) showed an objective response in only two of 37 patients treated.

PATIENTS AND METHODS

Sixteen patients with advanced breast cancer were admitted to the study. Five patients were premenopausal, one was menopausal (within 2 years of the menopause), and ten were postmenopausal.

Only four patients had a disease-free interval of > 2 years following their first treatment. Ten patients experienced a first disease-free interval of < 1 year. Uncontrolled disease was present in lymph nodes in three patients, in soft tissue in seven, and in the bones in 15. The disease at the primary site was controlled in all of the patients.

The experience of these patients with previous therapy for uncontrolled disease is shown in table 1.

TABLE 1.—Response to previous therapy

Therapy	Response*
Hormone	
Oophorectomy	2 CR/4
Hypophysectomy	1 CR/1
Estrogen	0/5
Antiestrogen	0/14
Cytotoxic	2 PR/4

*Values = No. of patients who responded/No. of patients treated; CR = complete response, PR = partial response.

The patients were given 560 mg of Estracyt/day orally in two divided doses. The minimum duration of therapy was 1½ weeks; the maximum duration was 6 months.

Symptomatic as well as objective changes were taken into account in the evaluation of response to Estracyt therapy. This was considered necessary because symptomatic response precedes objective response and because, in very advanced or aggressive bony disease, modification following successful therapy may not be observed because relapse has occurred before recalcification can be seen. A symptomatic improvement had to be seen for at least 2 months to be considered significant.

RESULTS

Using the above criteria for evaluation, two patients achieved partial symptomatic remissions, and one of these two had a partial objective remission.

Of the six patients whose cancer was diagnosed during or before the menopause, one showed a response to Estracyt. Similarly, only one of the ten patients whose cancer was diagnosed after the menopause showed a response.

Nausea only occurred in two patients and was easily controlled by antiemetics. When two patients with biochemical evidence of liver disease started Estracyt therapy, the liver became painful and tender. One patient became jaundiced. The jaundice and pain regressed when Estracyt was discontinued. The second patient died of advanced breast

[1]Received June 4, 1981; revised Oct 19, 1981; accepted Oct 29, 1981.

[2]Regional Radiotherapy Centre, Newcastle General Hospital, Newcastle Upon Tyne, England.

*Reprint requests to: Dr. P. J. D. K. Dawes, Regional Radiotherapy Centre, Newcastle General Hospital, Westgate Rd, Newcastle Upon Tyne NE4 6BE, England.

cancer while taking Estracyt, without any improvement in the hepatic symptoms and signs.

Marrow depression was not seen in any patients. Except for two patients with evidence of liver metastases, no changes were seen in the biochemical profiles.

DISCUSSION

Only one patient showed an objective remission with Estracyt, and one other showed a symptomatic improvement. These findings are in direct contrast to those of Alexander et al (4), who described 17 objective responses in 44 patients treated with a lower dose of Estracyt. Five patients in our study died within 1 month of receiving Estracyt and would have been regarded as unevaluable by Alexander et al. Even allowing for this, only one of 11 patients in our series had an objective response. This finding is more in keeping with the EORTC study, which showed an objective response in only two of 37 patients treated.

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Phase II Evaluation of Vindesine for Patients With Advanced Colorectal Carcinoma[1,2]

Clinical Trials Summaries

J. H. Saiers,[3,4,*] M. Slavik,[4] and D. R. McKinney[3]

Vindesine, a semisynthetic vinca derivative, has been tested using several dose schedules and appears to have definite antitumor activity in advanced malignancies such as acute leukemia, malignant lymphomas, and lung cancer (1,2). Therefore, a phase II vindesine study was begun in patients with advanced colon carcinomas. Histologic evidence of neoplastic disease with measurable metastatic lesions, a life expectancy > 1 month, normal liver and renal functions, adequate bone marrow reserve, no edema, and adequate hydration were required. Previous irradiation or chemotherapy was allowed, with a 4-week interval after previous chemotherapy.

Vindesine was administered iv on 2 consecutive days and repeated at 7- and 10-day intervals, depending on bone marrow recovery and toxic effects. Patients were given 2 mg/m² on Day 1 and 1 mg/m² on Day 2. This dose schedule, based on the known pharmacokinetics of vindesine (1), was predicted to achieve prolonged plasma concentrations of the drug for > 48 hours (2). An adequate trial was considered

[1]Received June 22, 1981; revised Oct 19, 1981; accepted Oct 29, 1981.

[2]Supported in part by Public Health Service grant CA-21074 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Hematology/Oncology Section, Veterans Administration Medical Center, Albuquerque, NM.

[4]Medical Oncology Division, Cancer Research and Treatment Center, University of New Mexico, Albuquerque.

Reprint requests to: Dr. J. H. Saiers, Hematology/Oncology Section, Veterans Administration Medical Center, 2100 Ridgecrest Dr, SE, Albuquerque, NM 87108.

TABLE 1.—Patient characteristics

Patient No.	Sex	Age (yrs)	Previous therapy	Hematologic toxicity nadirs: Wbc count (cells × 10^3/mm³)	Hematologic toxicity nadirs: Platelet count (cells × 10^3/mm³)	Response*	No. of courses
1	M	69	5-FU, Me-CCNU,† Baker's antifol	3.9	124	PD	4
2	M	60	High-dose methotrexate, 5-FU	1.4	303	PD	3
3	M	66	5-FU, Me-CCNU	0.2	152	PD	3
4	M	49	Vincristine, 5-FU, Me-CCNU	2.6	176	PD	1
5	M	66	Me-CCNU, 5-FU	2.5	236	PD	1
6	M	59	None	1.5	91	PD	4
7	M	70	Mitomycin, 5-FU	2.0	207	PD	3
8	M	66	Methotrexate, 5-FU	5.9	340	SD	8
9	M	52	5-FU	0.9	120	SD	8
10	F	69	5-FU, Me-CCNU, radiation	8.8	312	PD	1
11	M	45	5-FU	5.6	327	RT	1
12	M	70	5-FU, Me-CCNU	4.2	278	RT	2
13	M	57	5-FU	5.0	218	PD	5
14	M	50	5-FU, mitomycin, spirogermanium	3.1	317	PD	4
15	M	54	5-FU, Me-CCNU, radiation	0.9	70	SD	7

*PD = progressive disease; SD = stable disease; RT = refused treatment.
†Me-CCNU = methyl-CCNU.

to be three courses of vindesine or evidence of rapidly progressing disease.

The dose was decreased to 1 mg/m^2 on Day 1 and 0.5 mg/m^2 on Day 2 if leukopenia < 4000 cells/mm^3 or thrombocytopenia < 100,000 cells/mm^3 occurred. The initial dose was decreased 50% if serum bilirubin was > 2 mg/100 ml or if the patient had received < 4000 rad of radiation to the pelvis or spine.

The study involved 15 patients (table 1). Prior treatment included 5-FU in 14, nitrosoureas in six, a variety of chemotherapeutic agents in six, and radiation in two.

Four patients received one dose of vindesine; three of these had obvious disease progression, and the fourth refused further therapy. One patient refused therapy after two courses. The other patients received three to eight courses. No patients responded to treatment. Three had stable disease (< 25% regression of all measurable disease not qualifying for improvement) lasting 2–5 weeks.

Most patients tolerated the drug well. Three patients had mild to moderate nausea and vomiting, one had significant alopecia, one had anorexia, and one had numbness and tingling of the fingers. Marrow toxicity was the dose-limiting factor. The wbc count nadirs were 200–8800/mm^3 (mean, 3200). Plateletlet count nadirs were 70,000–380,000/mm^3. There was one possible drug-related death in a patient who developed septicemia with a wbc count of 200/mm^3 and subsequently died, although his wbc count returned toward normal before his death. This patient also had far-advanced disease at the time of septicemia.

We conclude that vindesine given in this dose schedule had no activity in previously treated patients with metastatic adenocarcinoma of the colon. Our results confirm those reported previously (3) using a weekly dose schedule.

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Phase II Trial of Thioproline in Advanced Epidermoid Head and Neck Tumors [1]

Clinical Trials Summaries

Francesco Boccardo,* Angelo Barbieri, Luciano Canobbio, Domenico Guarneri, Marco Merlano, and Riccardo Rosso [2,3]

Thioproline has recently been reported to restore contact inhibition of HeLa cells in culture and to induce reverse transformation of tumor cells to normal cells (1,2). Although not active in experimental tumors, it has been found to be highly effective against epidermoid tumors in man (2).

In view of the very exciting preliminary findings reported, a phase II study in advanced head and neck tumors has been undertaken at our institute.

MATERIALS AND METHODS

Twenty-two consecutive patients with epidermoid locally advanced head and neck tumors were treated with thioproline at a dose schedule of 40–60 mg/kg/day for 5 consecutive days every 3 weeks. Patient characteristics are shown in table 1.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Median age in yrs (range): 58 (36–73)	
Sex	
Male	19
Female	3
Median initial performance score (range): 1 (0–3)	
Primary tumor sites	
Oropharynx	7
Hypopharynx	1
Larynx	3
Nasal sinus	2
Oral cavity	7
Lower lip	1
Primary tumor size	
T_0–T_2	9
T_3–T_4	13
Nodal status	
N_0–N_1	12
N_2–N_3	10
Previous treatment	
None	8
Radiation therapy with or without chemotherapy	6
Surgery + radiation therapy with or without chemotherapy	8

RESULTS

Two patients died from early disease progression and one died from heart failure. Two patients were lost to followup. Therapy was discontinued in one patient due to drug toxicity. Thus, 16 patients who had received three therapy cycles were evaluable for response.

No objective response was observed in seven patients without prior treatment. Of nine patients with previous radiotherapy with or without chemotherapy, one showed a minor response (25% tumor shrinkage) lasting 4 weeks. This patient had completed radiotherapy 25 days before receiving thioproline and, most probably, the radiotherapy rather than the drug produced the improvement.

Fifty-five therapy cycles were considered for toxicity. Serious neurotoxic effects were encountered in four patients. One patient, aged 73, had seizures and coma during the first therapy cycle, while three patients had confusion and frank psychosis with paranoid delusion in the course of the third cycle. In all patients the neurologic symptoms resolved after therapy was discontinued. All patients were examined for neurologic disorders and all appeared to be normal.

[1]Received Aug 18, 1981; revised Nov 23, 1981; accepted Dec 2, 1981.

[2]Divisione ORL Ospedali Civili, Istituto Scientifico per lo Studio e la Cura dei Tumori, Genoa, Italy.

[3]We thank Dr. Giorgio Vadali for reviewing the manuscript, and Dr. Marc E. Lippman for his helpful suggestions.

**Reprint requests to:* F. Boccardo, MD, Divisione ORL Ospedali Civili, Istituto Scientifico per lo Studio e la Cura dei Tumori, Viale Benedetto XV, 10, 16132 Genoa, Italy.

CONCLUSIONS

Our results do not confirm the findings initially reported by Brugarolas and Gosalvez (2) in spite of the similar drug dose and schedule employed. In addition, serious neurologic toxic effects were encountered in four patients. Both neurotoxicity and lack of efficacy have been noted by other investigators (3,4). In view of the disagreement with regard to efficacy and of the serious neurologic toxicity, we do not recommend thioproline as an antineoplastic drug in man.

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Vindesine-Associated Angina and ECG Changes

Vindesine is a semisynthetic alkaloid derived from the vinca alkaloid vinblastine, with a spectrum of activity similar to that of vincristine. Less neurotoxicity is seen with vindesine than with vincristine, and the dose-limiting toxic reaction is leukopenia (1,2). Although myocardial infarction has reportedly been associated with both vincristine and vinblastine (3–5), acute transient ischemic changes have not been reported. We describe three patients receiving vindesine who developed acute chest pain and ECG changes suggestive of acute myocardial ischemia.

CASE REPORTS

A 58-year-old white male with malignant melanoma originating in the left shoulder region and with metastasis to the left supraclavicular lymph nodes had his third monthly course of vindesine (2.0 mg/m²/day for 5 days by continuous infusion); on the fourth day of the chemotherapeutic regimen he developed substernal chest pain, shortness of breath, and diaphoresis. The ECG revealed inverted T waves in the precordial leads, which had not been seen in pretreatment ECGs and were consistent with anterior lateral ischemia. Serial LDH and CPK levels were within normal limits. Aspirin and sulfinpyrazone were started. The next day, the patient was free of chest pain and the ECG returned to normal.

One month later, the patient received his fourth course of vindesine at a decreased dose of 1.8 mg/m² for 5 days. ECG changes were observed on the third day in the inferior wall leads. Four hours later no signs of ischemia were apparent on ECG. The patient was free of chest pain during the episode. On the fifth day of chemotherapy, the same transient ECG changes were documented and persisted throughout the day. One week later the ECG changes had normalized.

The second patient, a 65-year-old man with stage IIIA malignant melanoma, was treated with 2.0 mg/m²/day of vindesine for 5 days by continuous iv infusion. This patient had a significant history of atherosclerotic hypertensive vascular disease and coronary artery disease and had undergone a triple-bypass procedure 7 years prior to chemotherapy. He had been free of chest pain for the past 7 years. On the fifth day of chemotherapy, the patient developed severe chest pain, shortness of breath, diaphoresis, and nausea. The ECG showed an acute change suggestive of an inferior wall ischemia, with ST elevation in leads II, III, and AVF and with ST depression in the anterolateral leads. Serial CPK and LDH levels were within normal limits. All symptoms resolved within 24 hours and an ECG taken 1 week later was normal.

Patient 3 is a 57-year-old man with adenocarcinoma of the sigmoid colon and metastases to the liver. He was treated with intra-arterial infusion into the liver, consisting of 3 mg/m² of vindesine over 24 hours, 10 mg/m² of mitomycin, and 600 mg/m²/day of 5-FU for 5 days. On the fourth day of treatment, the patient developed acute chest pain, with ECG changes consisting of ST-segment elevation in the inferior and anterior leads (fig 1). All cardiac enzyme levels were within normal limits. The patient was asymptomatic within 24 hours. Subsequent ECGs resolved to normal (fig 2). One month later, the patient received his second course of chemotherapy at the same doses. On the fourth day, he reported moderately severe anterior chest pain that subsided in several hours. ECG changes consistent with those seen during the first course of chemotherapy developed. A followup ECG taken 4 weeks later was normal.

DISCUSSION

Toxic effects reported in clinical investigation of vindesine include myelosuppression (primarily leukopenia), peripheral neuropathy, paralytic ileus, alopecia, and phlebitis (1,2). So far, vindesine has not been reported to induce cardiac ischemia.

The other vinca alkaloids vincristine and vinblastine have been reported to be associated with myocardial infarction in cancer patients (3–5), but these reports are different from our report of patients with transient ischemia and changes suggesting an event of coronary spasm related to chemotherapy. In two of the patients (Nos. 1 and 3), no other causes contributing to acute cardiac ischemia could be identified. They did not have a previous history of ischemic heart disease, they had normal blood pressure and normal pulse on the day of the ischemic event, and though the third patient (No. 2) had a history of coronary heart disease, he had been free of any symptoms since his coronary bypass surgery 7 years before this event.

All three patients had an acute episode of chest pain, shortness of breath, and diaphoresis associ-

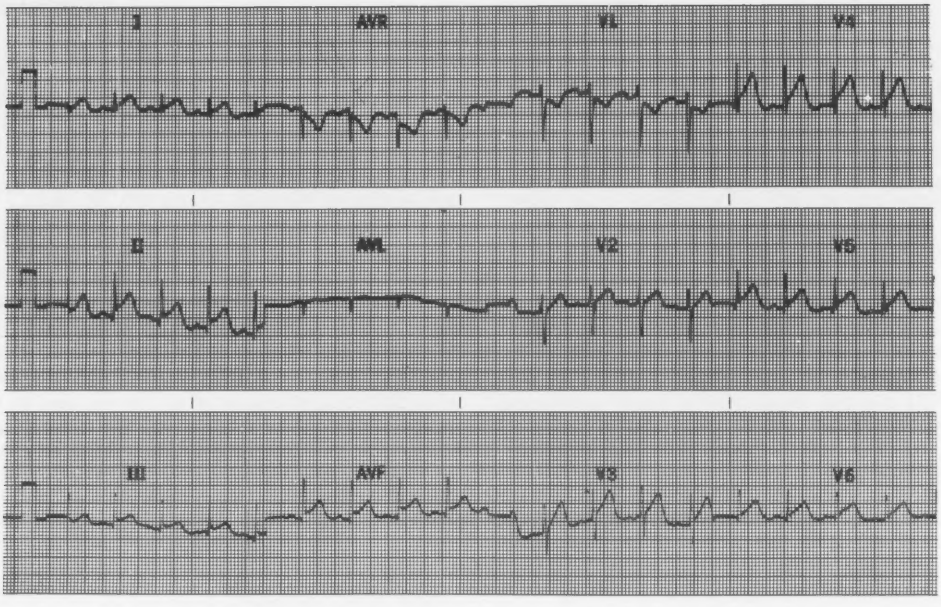

FIGURE 1.—ST elevation in leads I, II, III, AVF, and V_3–V_6 during episode in patient 3.

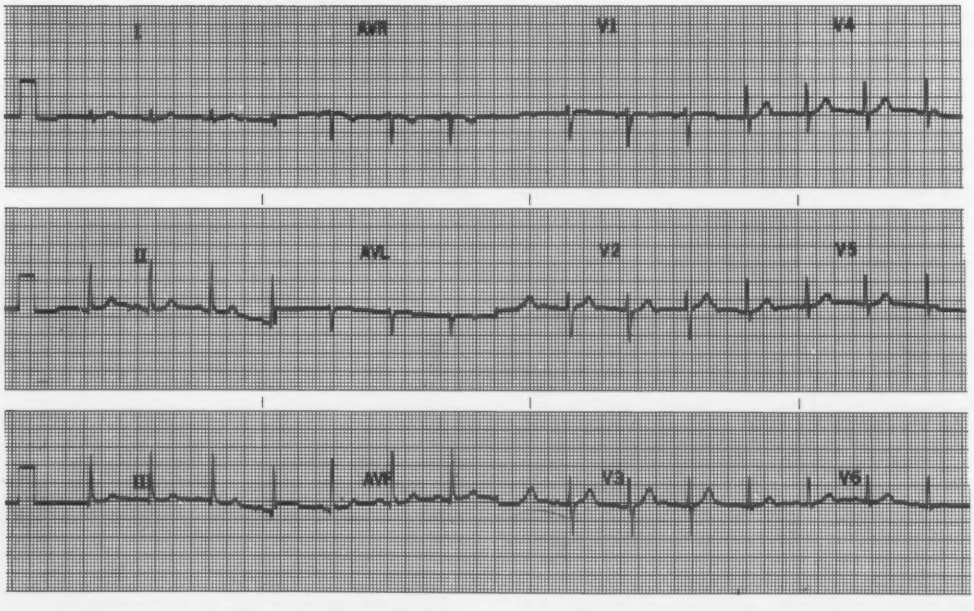

FIGURE 2.—Normal ECG taken 10 days after episode in patient 3.

ated with ECG changes. The symptoms resolved within 24 hours and the ECGs read as normal within 10 days of the episode.

In two patients who were exposed again to the drug (patients 1 and 3), ECG changes were noted again, and one of the patients had a recurrent episode of chest pain. Thus, the acute ischemia does not seem to be incidental but rather related to the vindesine. In patients 2 and 3 the ECG changes resemble those seen in Prinzmetal's angina, with acute ST-segment elevation and return to normal after several hours. An acute coronary spasm is the cause of this type of angina and is probably the cause of the acute cardiac ischemia in our patients. A release of vasoconstricting substance induced by vindesine may be the cause of this event. One such substance may be the serotonin released from activated platelets.

In recent years, calcium channel blocking agents have been used in the treatment of coronary spasms (6). Nifedipine and verapamil may be useful in the treatment of such events in patients with acute cardiac ischemia complicating vinca alkaloid chemotherapy.

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Richard S. Yancey[1]

Department of Pharmacy

Moshe Talpaz [2,*]

Department of Developmental Therapeutics

The University of Texas System Cancer Center
M. D. Anderson Hospital and Tumor Institute
6723 Bertner Ave
Houston, TX 77030

August 3, 1981

[1]Also affiliated with Texas Southern University School of Pharmacy, Houston.

[2]We thank Colleen M. Hubona for editorial assistance in the preparation of this manuscript.

*To whom reprint requests should be sent.

Contamination of Marihuana Cigarettes With Pathogenic Bacteria—Possible Source of Infection in Cancer Patients[1]

There is widespread interest in the use of cannabis or its active component, delta-9-tetrahydrocannabinol (THC), to alleviate the nausea and vomiting associated with cancer chemotherapy or radiation therapy. Most research studies have used oral delta-9-THC, but government-grown and -standardized marihuana cigarettes have also been used (1). "Legal" marihuana represents only a small proportion of the marihuana used by cancer patients; reports of use of illicit ("street") marihuana are widespread. In addition, 32 states now permit the controlled therapeutic use of marihuana.

Many cancer patients are immunosuppressed and have an increased risk of infection. This is particularly true of patients receiving intensive chemotherapy and radiation therapy. In studies performed as part of the UCLA THC Project, we have utilized marihuana cigarettes (supplied by the National Institute on Drug Abuse [NIDA]) in patients undergoing bone marrow transplantation. These patients receive high doses of immunosuppressive drugs and radiation, followed by transplantation of bone marrow from a histocompatible donor (2). They experience considerable nausea and vomiting from the therapy and may be unresponsive to standard antiemetics such as prochlorperazine (Compazine), trimethobenzamide (Tigan), and thiethylperazine (Torecan). In these patients, gastrointestinal mucosal denudation makes oral absorption of drugs erratic. Additionally, these patients cannot receive rectal suppositories or im injections because of profound decreases in granulocytes and platelets. As a result of these considerations, we chose to administer delta-9-THC via the inhaled route with the use of marihuana cigarettes.

Because these patients are at a high risk for infection, we performed bacteriologic studies of several lots of government-provided marihuana cigarettes. Substantial numbers of potentially pathogenic gram-negative bacteria were found. These bacteria could be eliminated by sterilization with ethylene oxide or radiation, without adversely affecting the delta-9-THC content of the cigarettes.

MATERIALS AND METHODS

Marihuana cigarettes were randomly selected from each of three separate lots[2] of government-grown cigarettes (2.02% THC) provided by the NIDA (under the auspices of FDA-IND-12,585) for use in clinical trials. Intact cigarettes were introduced into cul-

ture bottles containing trypticase soy broth and incubated at 37°C for 24 hours. The broth was then distributed to culture plates containing blood agar with or without polymyxin-B and McConkley media. Culture plates were incubated for 24–72 hours at 37°C and bacterial colonies were identified by routine microbiology techniques and by Gram stain. Cultures were performed in duplicate on six randomly selected cigarettes.

In some experiments we attempted to sterilize the marihuana cigarettes. Six cigarettes were individually sealed in plastic pouches. One half of the pouches were sterilized using ethylene oxide; the remaining cigarettes served as controls. This sterilization technique involved ethylene oxide (12%) and dichlorodifluoromethane (88%) at a controlled humidity of 45%, a temperature of 140°F, and a pressure of 8.5–10 psi. The cigarettes were sterilized for 4.5–5 hours and aerated for 12 hours prior to testing.[3] The sterilized and control cigarettes were then processed for bacterial cultures as indicated. In separate experiments, high-dose cobalt-60 radiation was delivered to two cigarette specimens at doses ranging from 100 to 20,000 Gray. Ethylene oxide-treated, irradiated, and control cigarettes were analyzed for delta-9-THC content by gas chromatography techniques.

RESULTS

All six marihuana cigarettes studied contained substantial numbers of *Klebsiella pneumoniae*, *Enterobacter agglomerans*, group D *Streptococcus* (enterococcus), *Bacillus* species, and *Enterobacter cloacae*. The cigarette samples treated with ethylene oxide and those treated with high-dose radiation (15,000–20,000 Gray) were bacteria-free. Neither sterilization technique altered the delta-9-THC content, which remained at 2.02% for all cigarettes.

DISCUSSION

We have demonstrated viable, potentially pathogenic bacteria in government-grown marihuana cigarettes supplied for investigative use in the prevention of chemotherapy- and radiation-induced nausea and vomiting.

The clinical implications of our findings are unclear. Lung infections rarely develop in healthy individuals who inhale air contaminated by bacteria because of the enormous capacity of the normal lung to inactivate bacteria. This may not apply to cancer patients, who may receive corticosteroids, cytotoxic chemotherapy, and radiation, all of which impair the antimicrobial defenses of the lung and increase susceptibility to infections.

Antibacterial defenses of the lung may be further compromised in patients who smoke marihuana; the gas phase of marihuana smoke has been shown to impair the intrapulmonary inactivation of bacteria (3). This effect is thought to be related to decreased phagocytic capacity of alveolar macrophages. Furthermore, we and others have reported that habitual smoking of cannabis may cause physiologic and histologic abnormalities of the pulmonary airways (4). These may lead to impaired clearance of particles and debris from the airways and predispose to infection.

It is not known whether the bacteria that contaminate marihuana cigarettes survive the heat of pyrolysis that develops during smoking. It is also unclear whether the bacteria are delivered in large enough numbers to the distal airways and alveoli to cause respiratory tract infection. In this regard, there is one recent report of *Aspergillus* pneumonitis in a young patient with chronic granulomatous disease who smoked several pipefuls of marihuana, a culture of which grew *Aspergillus fumigatus* (5), and another report of *Aspergillus*-contaminated marihuana cigarettes (6).

We found that sterilization of these cigarettes with ethylene oxide and/or radiation could completely eliminate bacterial contamination without adversely affecting delta-9-THC content. No determination of the effect of radiation on the taste of the cigarettes was made. High-temperature sterilization techniques such as autoclaving and microwaving were avoided since high temperatures are reported to cause pyrolytic transformation products.

In view of the high mortality associated with pulmonary infection in severely immunosuppressed patients, it may be useful to consider the sterilization of cigarettes when they are used in cancer patients receiving high doses of anticancer drugs and radiation.

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J. Thomas Ungerleider
Therese Andrysiak
Donald P. Tashkin
Robert Peter Gale [4,5]

Departments of Psychiatry and Medicine
UCLA School of Medicine
The Center for the Health Sciences
Los Angeles, CA 90024

September 11, 1981

[1]Supported in part by Public Health Service grants CA-21451, CA-23175, CA-18688, and CA-12800 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services; The Louis and Harold Price Foundation; The UCLA Jonsson Comprehensive Cancer Center; The Jewish Communal Fund of New York; and Mr. Charlie Roberts.

[2]Cigarettes were selected from NIDA THC cigarette lot Nos. 2055–166–1120, 1121, and 1122 (crop year, 1978).

[3]Analysis of placebo THC cigarettes for residual freon after 12 hours of aeration at a temperature of 130°F revealed 11 and 13 ppm freon in two separate samples.

[4]Dr. Gale is a scholar of the Leukemia Society of America.

[5]We thank Kenneth Davis (Research Triangle Institute) for performing the delta-9-THC analysis, James Whitbourne (Sterilization Technical Services, Inc) for performing the freon analysis of placebo marihuana cigarettes, and the staff of the UCLA Transplantation Biology Unit for calling the potential problem to our attention and for cooperating in the clinical trials.

Cytarabine in Chronic T-Cell Neoplasms

Cytarabine (Ara-C) has been used in combination chemotherapy for lymphomas and chronic lymphocytic leukemia, but the relative contribution of this drug to various combinations has been unclear. Ara-C has not been systematically evaluated in chronic T-cell neoplasms. Levi and Wiernik (1) mention one patient with cutaneous T-cell lymphoma (mycosis fungoides) who was treated with Ara-C and "responded" but no details were given. Stein (2) described a young man with pulmonary involvement by mycosis fungoides who responded to a combination of cyclophosphamide, vincristine, methotrexate, leucovorin, and Ara-C. The relative activity of the various drugs could not be assessed. Two patients were recently treated with Ara-C alone, with useful, albeit temporary, responses.

A 48-year-old woman with marked lymphocytosis, generalized lymphadenopathy, marked splenomegaly, and cutaneous infiltrates was shown to have chronic T-cell lymphocytic leukemia. Initial treatment with chlorambucil and prednisone was ineffective. Cyclophosphamide, vincristine, doxorubicin, bleomycin, and high-dose methotrexate with leucovorin rescue were also without benefit. However, a 72-hour continuous infusion of Ara-C produced a decrease in the patient's lymphocyte count from 300,000 to 7000 cells/mm^3. Her splenomegaly and lymphadenopathy regressed slightly. Cutaneous infiltrates regressed. Previously unexplained congestive heart failure improved as did renal function. With repeated infusions of Ara-C, symptomatic improvement continued for 2½ months, at which time the patient's wbc count began to increase again and thioguanine was added to her regimen.

A 55-year-old man with cutaneous T-cell lymphoma (mycosis fungoides) had biopsy-proven pulmonary involvement. He had previously been treated with cyclophosphamide, doxorubicin, vincristine, and prednisone, with a transient improvement in the appearance of the skin and chest X-ray. His disease then progressed, so he was treated with high-dose methotrexate and leucovorin rescue, with slight improvement in his skin but disease progression as indicated by X-rays. At that point, and in view of the response shown by the first patient, he was treated with repeated courses of Ara-C by continuous 48-hour infusion, resulting in marked improvement in his skin and a partial regression of the lung lesions. This response persisted for 2 months.

Although both patients had definite responses to Ara-C, it is not possible to say whether the T-cell origin of the diseases influenced the response. As noted above, other lymphoid neoplasms have been thought to be responsive to this drug. It is of interest, however, that Ohnuma et al (3) found Ara-C to be much more inhibitory against T-cells than B-cells in culture. Recently, Ara-C has been combined with doxorubicin in treating lymphoblastic lymphoma (4), which is generally of T-cell origin. Although more elegant approaches to therapy are becoming available (5), further evaluation of Ara-C in mycosis fungoides and other chronic T-cell neoplasms seems warranted.

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George A. Omura

Division of Hematology and Oncology
Department of Medicine
School of Medicine
University of Alabama
638 Zeigler Bldg
University Station
Birmingham, AL 35294

September 28, 1981

Enhanced Bleomycin Toxicity During Acute Renal Failure

Bleomycin is primarily excreted in the urine whether it is administered im or iv (1–3). Approximately 70% of the total dose is excreted in the first 24 hours (2). Renal dysfunction can significantly prolong bleomycin excretion (2,3), with an exponential increase in the serum half-life as the creatinine clearance decreases (2). For example, the half-life can be as long as 21 hours at a creatinine clearance of 10.7 ml/min, whereas at a clearance > 50 ml/min it is ordinarily 1–2 hours (3).

The toxic effects of bleomycin are primarily fibrosing alveolitis and mucocutaneous inflammation, which are cumulative and dose-related. Although this drug is excreted in the urine, it produces no renal toxicity. A recent report (4) of fatal bleomycin-induced pulmonary fibrosis occurring during renal failure suggests that reduced urinary clearance of bleomycin exposes the patient to unusual degrees of drug toxicity. We have observed a similar phenomenon of severe bleomycin toxicity in a patient who had poor renal function due to administration of cisplatin. This patient manifested severe cutaneous bleomycin toxicity and also had neurologic symptoms that may have been related to bleomycin.

The patient was a 58-year-old man diagnosed in February 1977 as having unresectable squamous cell carcinoma of the hypopharynx. Pretreatment cbc and serum chemistries were normal. The creatinine clearance was 120 ml/min. Pulmonary function including D_LCO was normal. He received 7.6 mg of vinblastine iv on Day 1, 15 units of bleomycin im on Days 1–7, and 220 mg (120 mg/m²) of cisplatin with mannitol diuresis on Day 8. He returned for his second course on Day 21. By then the patient had responded with at least a 50% reduction in the size of the original tumor. Hemoglobin was 9.6 g/dl, serum creatinine was 8.2 mg/dl, and creatinine clearance was 10 ml/min. Vinblastine was given again at a dose of 7.6 mg, and 15 units of bleomycin was given im on Days 1–6. Eight days after completing the bleomycin, the patient developed a maculopapular rash on the chest and arms. Subsequently, he developed pain in his fingers, elbows, and knees and hard nodules over the palms. Radiation therapy to the hypopharynx and neck was started on Day 30 but had to be stopped after a total of 360 rad because of development of pustular lesions on the tongue and palate. The patient also developed cerebellar signs with truncal ataxia and uncontrolled shaking. The serum creatinine decreased to 3.3 mg/dl by Day 50. The patient did not develop cough, rales, changes on chest roentgenogram, or deterioration in pulmonary function aside from a 20% reduction in the pretreatment diffusing capacity. Radiation therapy was restarted and completed in July 1977. The tumor was in complete remission at the end of radiation therapy. The skin reaction resolved slowly but left hyperpigmentation and markedly thickened skin over the hands and feet. There was sausage-shaped swelling of the fingers and toes.

In June 1978 the patient had no evidence of tumor but he appeared to have scleroderma of the hands and feet. The serum creatinine was 4.1 mg/dl and the creatinine clearance was 9 ml/min. Chest roentgenograms showed interstitial pneumonitis. The patient continued to have ataxia and developed episodes of unexplained loss of consciousness. Neurologic workup, including cranial computerized tomography, EEG, and lumbar puncture, was normal. Several weeks later the patient became comatose and died. Autopsy was not permitted.

This patient developed severe skin fibrosis resembling scleroderma and moderate interstitial pulmonary fibrosis from the bleomycin. He also developed unexplained neurologic symptoms possibly related to bleomycin. Although he received vinblastine, a known neurotoxin, it is unlikely that the small doses received caused this severe neurologic problem. Vinblastine is primarily excreted in the gut and would not be expected to reach toxic levels in the setting of renal failure. Like the patient who had fatal bleomycin pulmonary toxicity (4), our patient had deficient renal function due to cisplatin treatment, which occurred despite mannitol diuresis. Our patient developed scleroderma that persisted until he died 15 months later. Although not a life-threatening complication of his treatment, the severe cutaneous problem reiterates the point that renal function must be adequate when bleomycin is used, or severe toxic reactions can occur.

The ataxia, uncontrollable shaking, and subsequent coma have not been previously described as side effects of bleomycin. Of some interest is that a bleomycin analog, pepleomycin, has been reported

to produce ataxia in mice (5). Unfortunately, autopsy was not permitted, which would have allowed brain examination, but there was no evidence of metastasis on computerized tomography scan 1 month prior to death. Perhaps these findings were also related to previous prolonged high serum levels of bleomycin.

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David J. Perry
Raymond B. Weiss
H. Grant Taylor

Hematology Oncology Service
Walter Reed Army Medical Center
Washington, DC 20012

October 7, 1981

AMSA Therapy for Children With Lymphoblastic Malignancy

AMSA is an acriflavine derivative that inhibits DNA synthesis. Preliminary studies have suggested that AMSA is an active drug in patients with acute myelogenous leukemia (1–3) and acute lymphoblastic leukemia (ALL) (1–4). This report describes the response and toxic reactions observed in children with refractory ALL or lymphoblastic lymphoma who were treated with AMSA.

METHODS

Nine children 5–15 years of age with ALL or lymphoblastic lymphoma refractory to all standard chemotherapy, including doxorubicin, were entered in this study. Seven children had E-rosette-negative (E^-) ALL; two had E-rosette-positive (E^+) T-cell disorders (ALL and lymphoblastic lymphoma). AMSA was diluted in 5% dextrose in water and administered by iv infusion over 1–4 hours (median infusion time of 2 hours in five patients) daily for 5 days. In eight of the nine patients the total dose administered over the 5-day course was 450–675 mg/m^2 (median dose of 500 mg/m^2 administered to four patients). One patient with severe pre-existing liver disease received a cumulative dose of 175 mg/m^2 over 3 days. Prior to therapy, all patients with ALL had a bone marrow examination demonstrating leukemic relapse; the child with lymphoblastic lymphoma had a malignant pleural effusion and a large subcutaneous tumor. Studies to determine the toxicity of AMSA included serial determinations of blood cell counts along with measurement of serum bilirubin, SGOT, and creatinine.

RESULTS

Two of the nine children (22%) demonstrated a complete clinical resolution of disease after AMSA treatment. A 15-year-old boy with E^- ALL, who had persistent blast cells in the peripheral blood and bone marrow despite the administration of doxorubicin 2 weeks previously, attained a complete remission after AMSA therapy. The duration of unmaintained complete remission was 4 months. This patient had severe chronic hepatitis and responded to a relatively low dose of AMSA (175 mg/m^2 over 3 days). However, increased toxic reactions, particularly nausea and vomiting plus a transient increase in serum bilirubin from 1.2 to 3.3 mg/dl, were also noted. An 8-year-old boy with T-cell lymphoblastic lymphoma, who had developed progressive increase in a large subcutaneous tumor and malignant pleural effusion while on a combination chemotherapy regimen that included doxorubicin, demonstrated complete resolution of the tumor and the malignant pleural effusion. The effusion, however, began to reaccumulate 3 weeks later but temporarily resolved after additional treatment with AMSA. Five other children, although they did not achieve a complete response, had minor evidence of antitumor activity demonstrated by improvement in bone pain, disappearance of blast cells from the peripheral blood, and/or development of marrow hypoplasia. In all, seven of nine children (78%) had some evidence of antitumor activity from AMSA.

Toxic effects of AMSA in this dose schedule included moderate to severe stomatitis (three of nine patients), pain at the infusion site (five of nine), transient headaches (one of nine), nausea and vomiting (two of nine), and mild abnormalities in liver function when it had been normal prior to treatment (two of eight). Two children had increased hair loss after AMSA.

The myelosuppressive effects of this dose schedule of AMSA were difficult to quantitate in our patients who had granulocytopenia and thrombocytopenia secondary to leukemic relapse. All children, however, demonstrated a further decrease in wbc and platelet counts after AMSA therapy. In the child with lymphoblastic lymphoma who had normal pretreatment marrow reserve, a total wbc count nadir of 300/mm^3 and a platelet count nadir of 1000/mm^3 occurred 15 days after the start of AMSA therapy. The granulocyte and platelet counts, however, returned to normal by Day 24 of AMSA therapy.

DISCUSSION

Two of the nine children with advanced lymphoblastic malignancy achieved a complete remission after AMSA therapy. One of the complete responders had an E$^+$ lymphoblastic lymphoma. All of the children treated with AMSA were refractory to anthracycline therapy. This indicates that these classes of compounds are not completely cross-resistant in children with lymphoid malignancy.

The therapeutic effectiveness of AMSA in patients with acute leukemia may be very dose-dependent. Arlin et al (3) noted an improved response rate of leukemic patients receiving AMSA in doses of 1000 mg/m^2 compared to those receiving 500 mg/m^2, both given over 5 days. The administration of AMSA at this dose range, however, produces marked myelosuppression (4,5), and stomatitis becomes a major problem when AMSA is infused at doses of 125–150 mg/m^2/day over 5 days (4). All of our patients treated with AMSA who exhibited a response to treatment developed marked bone marrow hypoplasia, including the child with pre-existing liver disease who achieved a complete remission at a relatively low dose (175 mg/m^2 over 3 days). Since AMSA is metabolized in the liver, the response of this patient to a lower dose of AMSA may reflect altered pharmacologic clearance of the drug.

We conclude that the administration of AMSA in doses > 450 mg/m^2 over 5 days is associated with definite antitumor activity in children with lymphoblastic malignancy. The marked degree of marrow hypoplasia induced by this dose schedule of AMSA, however, may limit the ultimate ability to combine AMSA with other effective but very myelosuppressive agents.

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John J. Hutter, Jr

Department of Pediatrics

Frank L. Meyskens

Department of Medicine

University of Arizona Health Sciences Center
Tucson, AZ 85724

October 19, 1981

Allergic Reaction Following Administration of AMSA

We read with interest the report of Welt and his colleagues (1) describing the occurrence of an acute skin reaction following administration of AMSA. Due to the apparent rarity of this reaction, we report a similar case in an adult male treated for recurrent acute myelogenous leukemia (AML).

CASE REPORT

A 30-year-old Samoan male presented in January 1978 with recurrent fever, myalgias, gum bleeding, and a wbc count of 60,000/mm^3. A diagnosis of AML was confirmed by bone marrow biopsy. Following a course of doxorubicin and cytarabine, the patient entered into a complete remission documented by bone marrow aspiration.

The patient remained well until February 1981 when recurrent AML was diagnosed, based on peripheral blood smear and bone marrow findings. A complete remission was obtained after retreatment with cytarabine and doxorubicin combination chemotherapy.

In September 1981 the patient re-presented with recurrent AML. At that time, treatment was initiated with 240 mg of AMSA in 500 ml of 5% dextrose in water at a rate of 1 mg/minute. Following the start of his infusion, the patient noted burning at the site of his iv line and was observed to have

a pruritic, erythematous, nonurticarial rash on his arm only. At no time did the patient report nasal stuffiness, shortness of breath, wheezing, or other symptoms. His vital signs remained stable throughout the initiation of the infusion. Following administration of 50 mg of Benadryl iv, the patient appeared significantly better. For this reason, the infusion was reinitiated in the other arm. Shortly thereafter, the patient developed a generalized drug eruption unassociated with other systemic symptoms. The AMSA was discontinued at that time.

DISCUSSION

As previously reported, allergic skin reactions associated with AMSA therapy appear to be rare. No life-threatening systemic symptoms were encountered in our patient as was the case in the report of Welt et al (1). We cannot comment on the advisability of continuing therapy with AMSA following an episode of skin allergy only. In our patient the severity of the reaction appeared to worsen with subsequent administration of the drug, despite Benadryl therapy. Of interest is the possibility that these reactions may represent problems with our recent lot of drug rather than with the AMSA itself. We are currently investigating this possibility.

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Fred P. Rosenfelt
Barry E. Rosenbloom
Irwin M. Weinstein

Cedars-Sinai Medical Center
Los Angeles, CA 90048

October 19, 1981

ANNOUNCEMENTS

Eighth Annual Symposium on Diagnosis and Treatment of Neoplastic Disorders—Medical, Surgical, and Radiotherapeutic Aspects

Baltimore, Maryland, March 18–20, 1982

This symposium, presented by the Oncology Center of Johns Hopkins University, will focus on three major areas: new approaches in cancer treatment, therapy of specific malignancies, and management of the complications of cancer. The program will include mini-symposia on breast cancer, chronic myelocytic leukemia, Hodgkin's disease, and female germ cell tumors. Biologic response modifiers, hyperthermia, radioimmunoglobulin therapy, new anticancer drugs, and in vitro drug testing will also be highlighted. In recognition of the importance of total care of cancer patients, discussions of new approaches to viral infections, management of urologic complications, and psychologic management of cancer patients and their families have been scheduled. The course will conclude with three interesting cases at Grand Rounds and an overview on the management of ovarian cancer at Conjoint Clinic. The Hopkins faculty will be joined by distinguished guest lecturers. Liberal time will be allotted for informal exchange of ideas with the faculty.

The registration fee is $250.00, which includes social activities. Eighteen credit hours will be awarded by the AMA.

For further information, contact: Program Coordinator, Continuing Education, Turner Auditorium, Rm 22, 720 Rutland Ave, Baltimore, MD 21205 (301-955-5880).

First National Symposium on Psychoimmunology

New York, New York, April 24–25, 1982

Leading scientists and clinicians will review recent discoveries about the way the body's natural defenses against disease are influenced by stress and our response to it at a 2-day symposium on "Mind and Immunity" at the New York Academy of Medicine.

Participants will include Drs. Carolyn B. Thomas, Robert Ader, David McClelland, Marvin Stein, and Sandra Levy. Steven Locke, MD, will chair the symposium.

This program is intended for medical, behavioral, and mental health professionals and is approved for continuing education credits for physicians, psychologists, and other professionals. Tuition is $120.

For more information, contact: Institute for Psychosocial Study, 221 E 50th St, New York, NY 10022 (212-838-3122).

Twelfth Annual Neurosurgery Postgraduate Course and Sixth Annual Neurosurgical Nurses' Conference

San Francisco, California, June 3–4, 1982

This course will provide an update on selected, often controversial, topics in clinical neurosurgery. The lectures will be presented by distinguished visiting faculty including Drs. Fred Epstein, Irvin Kricheff, Leonard Malis, and Robert Spetzler. Selected faculty from the University of California at San Francisco will also participate. Cerebral and spinal neoplasms, cerebrovascular disease, and recent clinically relevant advances in the neurologic sciences will be covered in depth. In addition to the didactic sessions, there will be luncheon discussions between small groups of registrants and individual members of the faculty on a variety of neurosurgical problems.

This program is presented by the Department of Neurological Surgery of the University of California School of Medicine at San Francisco. It is sponsored by Extended Programs in Medical Education and will meet the criteria for 16 hours of ACCME/AMA/CMA Category 1 (formal) continuing credit for physicians. The program is also approved for nursing credit.

For further information, contact: Extended Programs in Medical Education, University

of California School of Medicine, Rm 569-U, Third and Parnassus, San Francisco, CA 94143 (415-666-4251).

Second International Symposium on Infections in the Immunocompromised Host

Stirling, Scotland, June 20–24, 1982

This symposium, sponsored by Stirling University, will provide a forum for presenting and discussing new concepts and findings related to infections in the compromised host. Guest speakers will present lectures in general sessions and workshops and poster sessions are also planned. The final registration date is March 15.

Accomodation is available on the Stirling University Campus. Pre- and post-symposium accomodation at the University is also available on application.

For further information, contact: Dr. Harold Gaya, Microbiology Department, Brompton Hospital, Fulham Rd, London SW3 6HP, England.

Urologic Cancer Course

Boston, Massachusetts, September 22–24, 1982

This intensive 3-day course, to be held at the Parker House Hotel, will provide an overview of the clinical and pathophysiologic basis for the diagnosis and treatment of patients with urologic cancer. The course will emphasize the multidisciplinary approach that constitutes the foundation of contemporary care for patients with urologic cancers. Principles derived from the disciplines of urologic surgery, medical oncology, and radiation therapy will be highlighted and incorporated into a comprehensive consideration of therapeutic practices. Although teaching will be primarily provided through lectures, panel discussions addressing major current controversies in urologic cancer will be an integral portion of the course. A comprehensive syllabus will be provided.

Guest faculty will include Drs. Lawrence H. Einhorn, Donald Skinner, Willet F. Whitmore, Jr, and Leonard Zinman. The Harvard faculty participants will include leaders in urologic cancer from the Sidney Farber Cancer Institute, Brigham & Women's Hospital, Joint Center for Radiation Therapy, Children's Hospital Medical Center, Beth Israel Hospital, and Massachusetts General Hospital.

The course fee is $325.00.

For further information contact: Harvard Medical School, Department of Continuing Education, Boston, MA 02115 (617-732-1525).

Position Vacancy: Director, Division of Cancer Treatment

The position of *Director of the Division of Cancer Treatment* is now vacant. This position is located within the National Cancer Institute (NCI), National Institutes of Health, Public Health Service, Department of Health and Human Services.

This is a civil service position in the Senior Executive Service (SES), with a salary range from $54,755 to $61,300 (currently limited to $58,500 per annum). Candidates may be eligible for Physician's Comparability Pay up to $10,000 per year. The individual selected, if not presently in the SES, must serve a 1-year probationary period. (Alternatively, candidates may be eligible for appointment in the Commissioned Corps of the US Public Health Service.)

The NCI develops the overall national strategy against cancer, coordinates federal government and nonfederal government cancer programs, and supports international programs of cancer research. NCI effort covers the spectrum of basic, applied, and developmental research. It encompasses the research thrusts of cause and prevention of cancer, detection and diagnosis, treatment and rehabilitation, and cancer biology. It also encompasses control interventions in prevention; detection, diagnosis, and pretreatment evaluation; and treatment, rehabilitation, and continuing care.

As Director of the Division of Cancer Treatment, the incumbent will be responsible for directing the NCI's major research effort related to the treatment of cancer. The Division

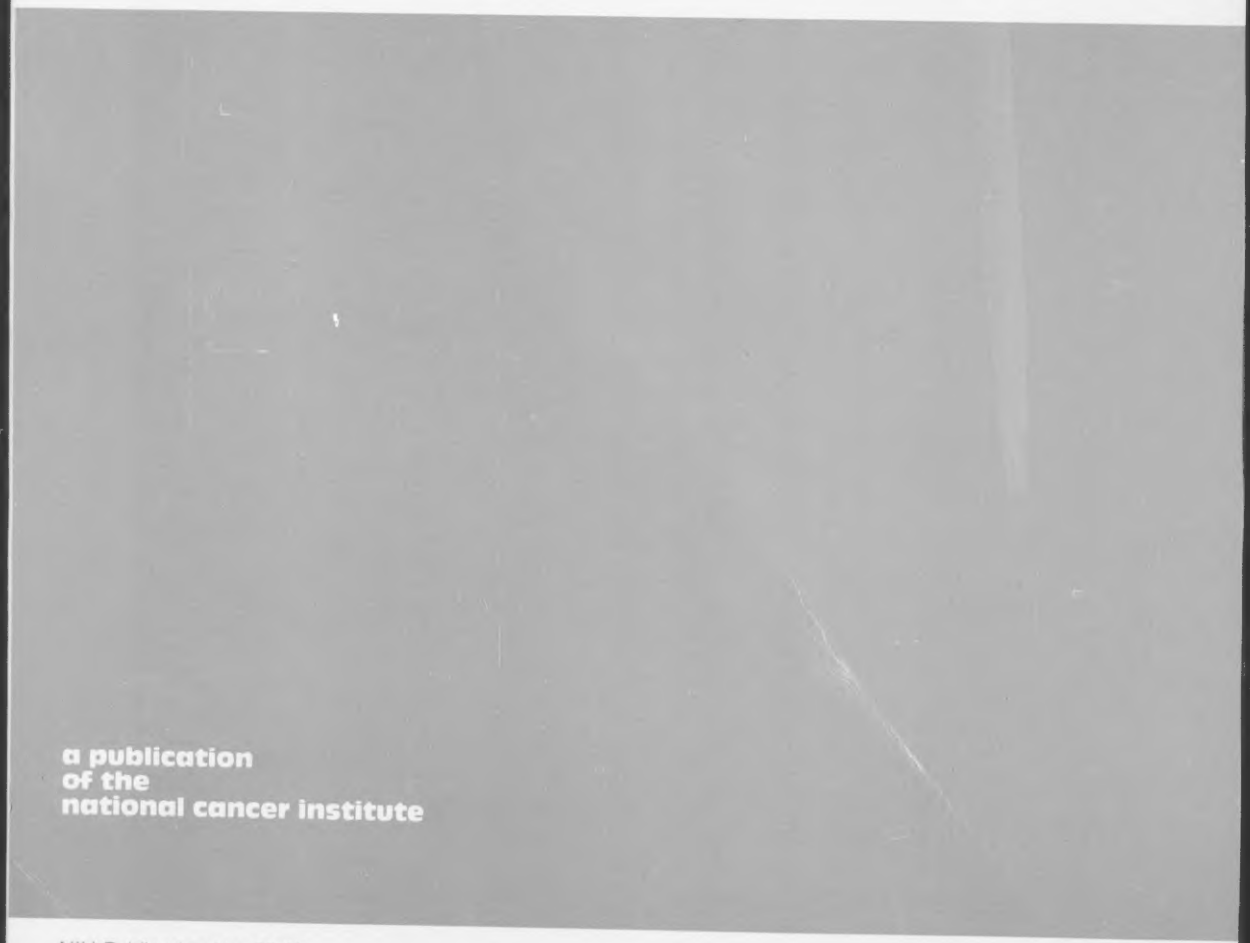
encompasses programs for intramural laboratory research. The Division also is responsible for extramural (grants, contracts, and cooperative agreements) activities related to its mission. It is anticipated that the Division will have a budget of approximately $275 million and approximately 473 full-time permanent personnel in fiscal year 1982.

The mandatory professional/technical qualifications for the position require that candidates: (1) meet the minimum educational requirements for Medical Officer, GS-602, or Health Scientist, GS-601, or equivalent combination of experience and training, as defined by the US Office of Personnel Management Handbook X-118 (Qualification Standards for Positions under the General Schedule), which is available in federal personnel offices; and (2) have research experience and knowledge of research programs in one or more areas related to the treatment of cancer. The managerial/executive qualifications are: experience in the leadership/management of a research program, demonstrating competence to assume leadership responsibilities in: (1) assuring that national and agency goals and priorities are considered in carrying out the functions and responsibilities of the organization; (2) establishing and maintaining relationships with key individuals and groups outside the organization; (3) directing and guiding programs and projects, including long-term and short-term planning and establishing objectives and priorities; (4) establishing procedures for the development and defense of the organization's budget and allocating fiscal and personnel resources to support program implementation; (5) developing and implementing procedures for monitoring program performance, including periodic analysis and evaluation; and (6) developing processes and overseeing activities to implement EEO objectives and to assure the fair and equitable employment of qualified persons.

Candidates who meet the mandatory requirements will be evaluated by the Search Committee on the following factors: scientific reputation/peer recognition, training and education, honors and awards, and the degree to which they exceed the minimally qualified level of the mandatory professional/technical and managerial/executive qualifications.

Applications (Personal Qualifications Statement, SF-171) are to be sent to Ms. Dreama Chapman, National Cancer Institute, Bldg 31, Rm 3A34, Bethesda, MD 20205. A current curriculum vitae and bibliography must accompany all applications. Applications of women and/or minority group members are especially solicited. The National Institutes of Health is an equal opportunity employer. Applications must be postmarked *no later than March 31, 1982.*

Additional information may be obtained from: Ms. Dreama Chapman, Supervisory Personnel Management Specialist, NCI (301-496-6862).



**a publication
of the
national cancer institute**

NIH Publication No. 82-22